# Cleavage sites of endoproteases on the a-chain of GPIb

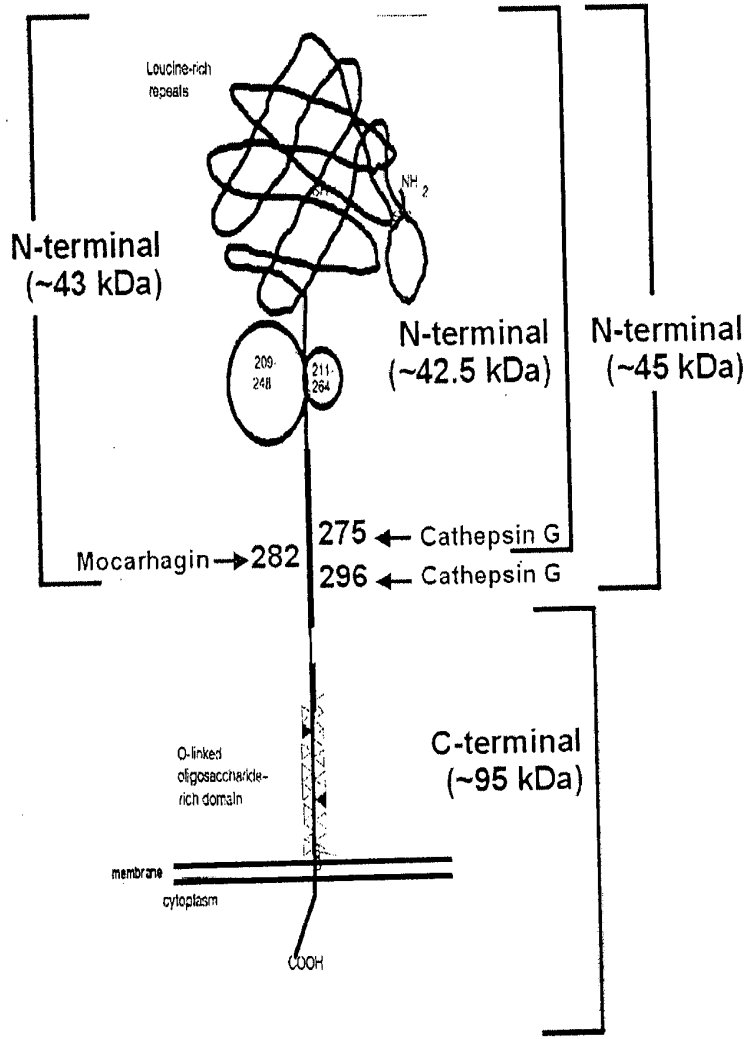
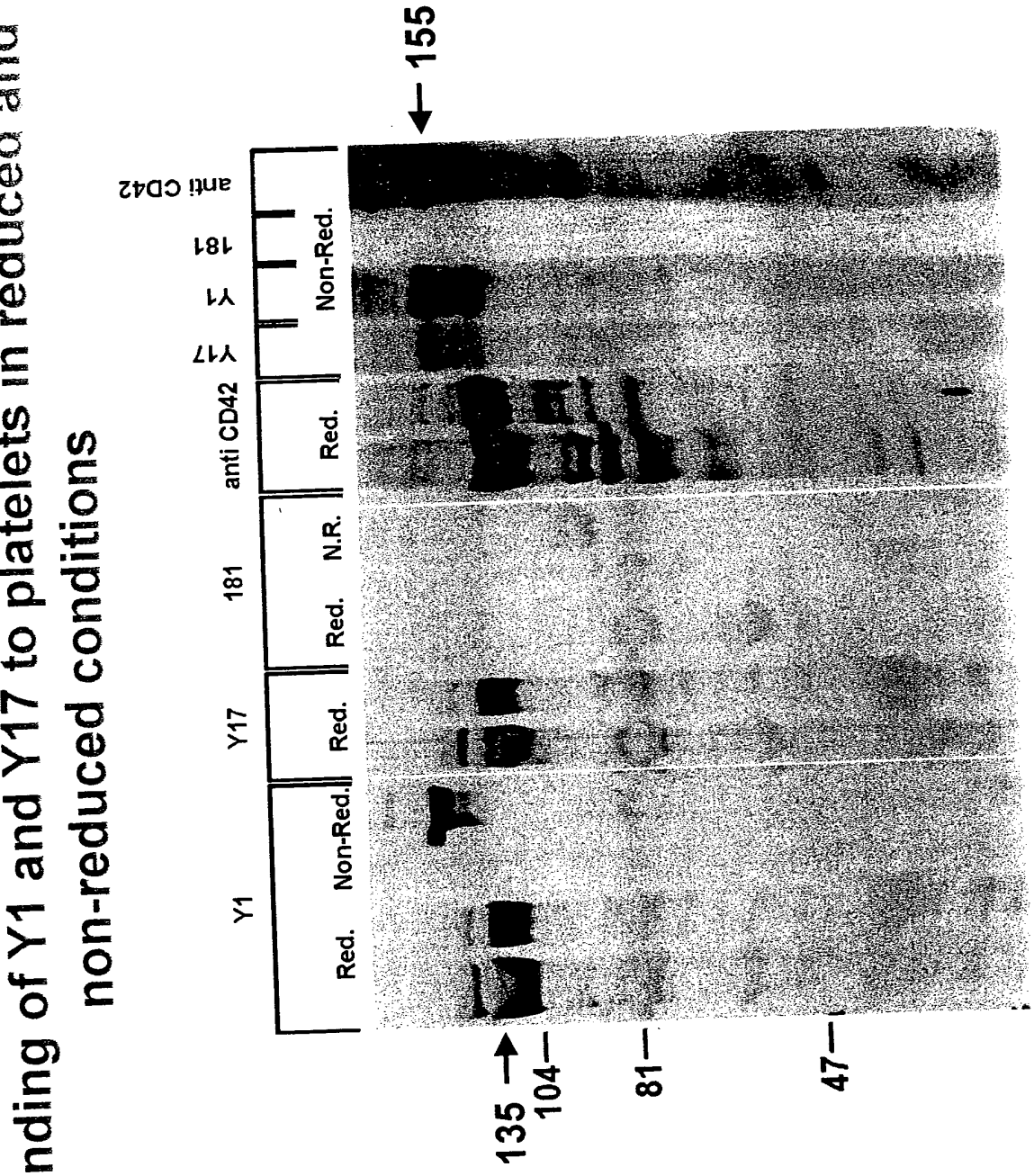



FIG. 1

#4

*FIG. 2*



# Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

| | Platelets/GC | KG1/RP-HPLC #4 |
|---|---|---|
| Rec: GP1b 1-340 | - | |
| GP1b 1-480 | - | |
| Glycanase: N | + | +++ |
| N+O | + | +++ |
| Proteases: Mocarhagin | ++ (~40kD) | - |
| O-Sialo Peptidase | ++ (~40kD) | - |
| Ficin | - | - |
| Trypsine | ++ (~40kD) | - |
| Elastase | ++ (~40kD) | ++ |
| Sulfatase (Aryl) | | -/+ |

*FIG. 3*

*FIG. 4*

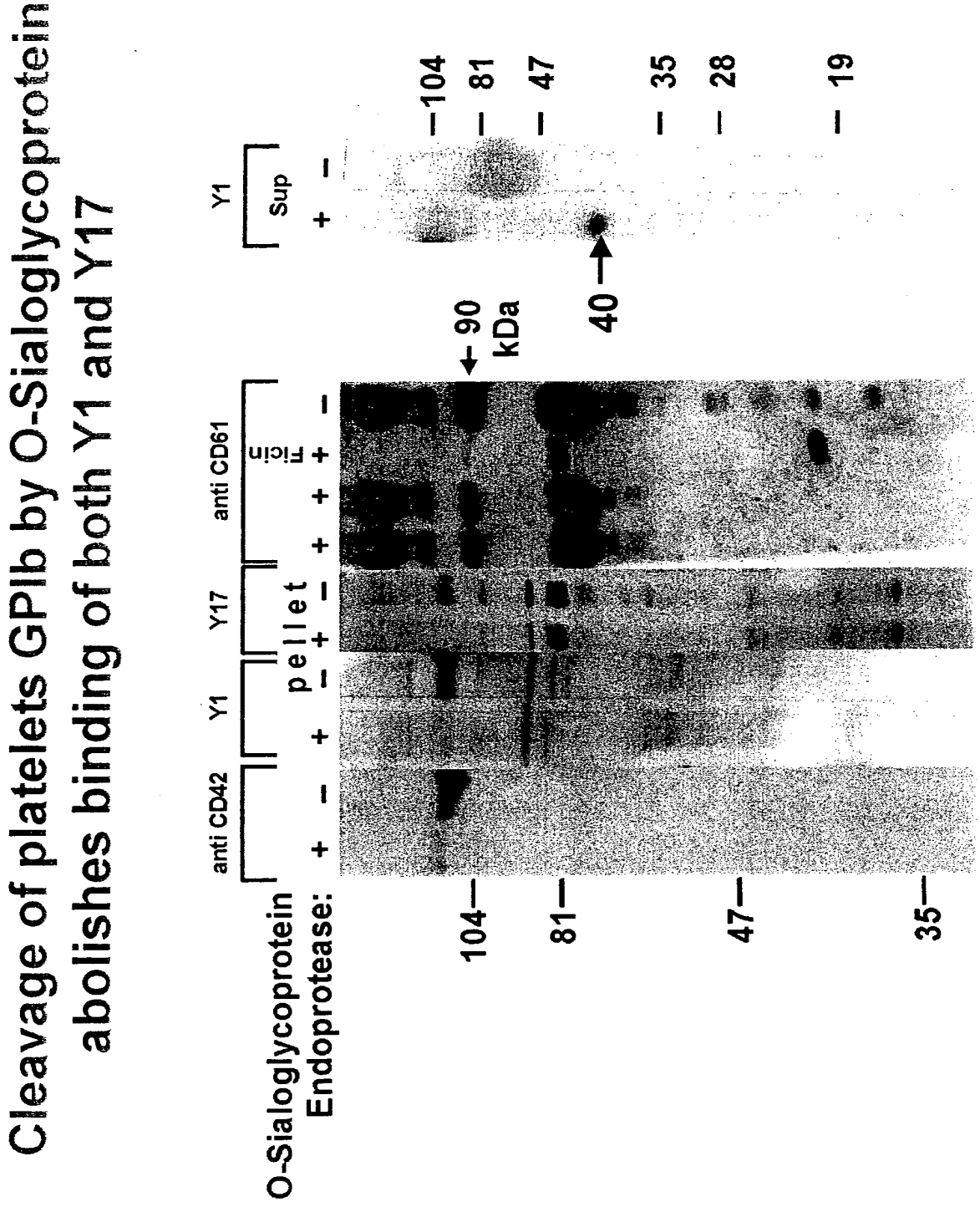


*FIG. 5*

Y1 and Y17 binds similar glycocalycin fragments after cleavage by O-Sialoglycoprotein Endoprotease

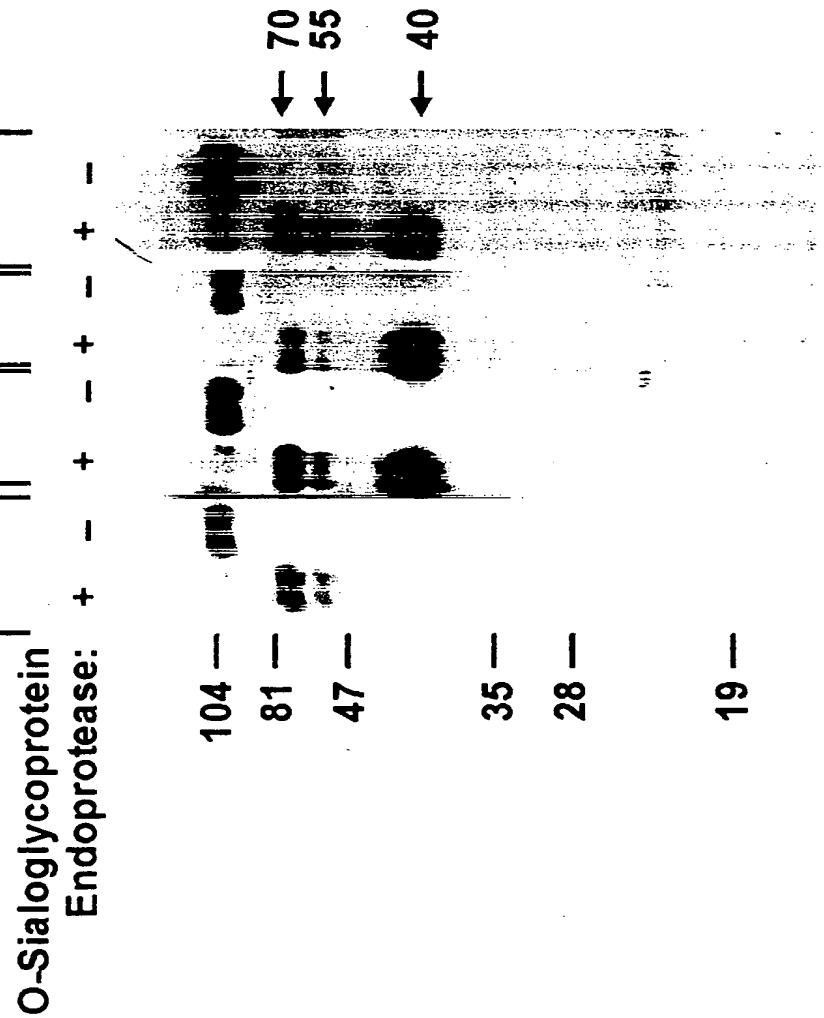


*FIG. 6*

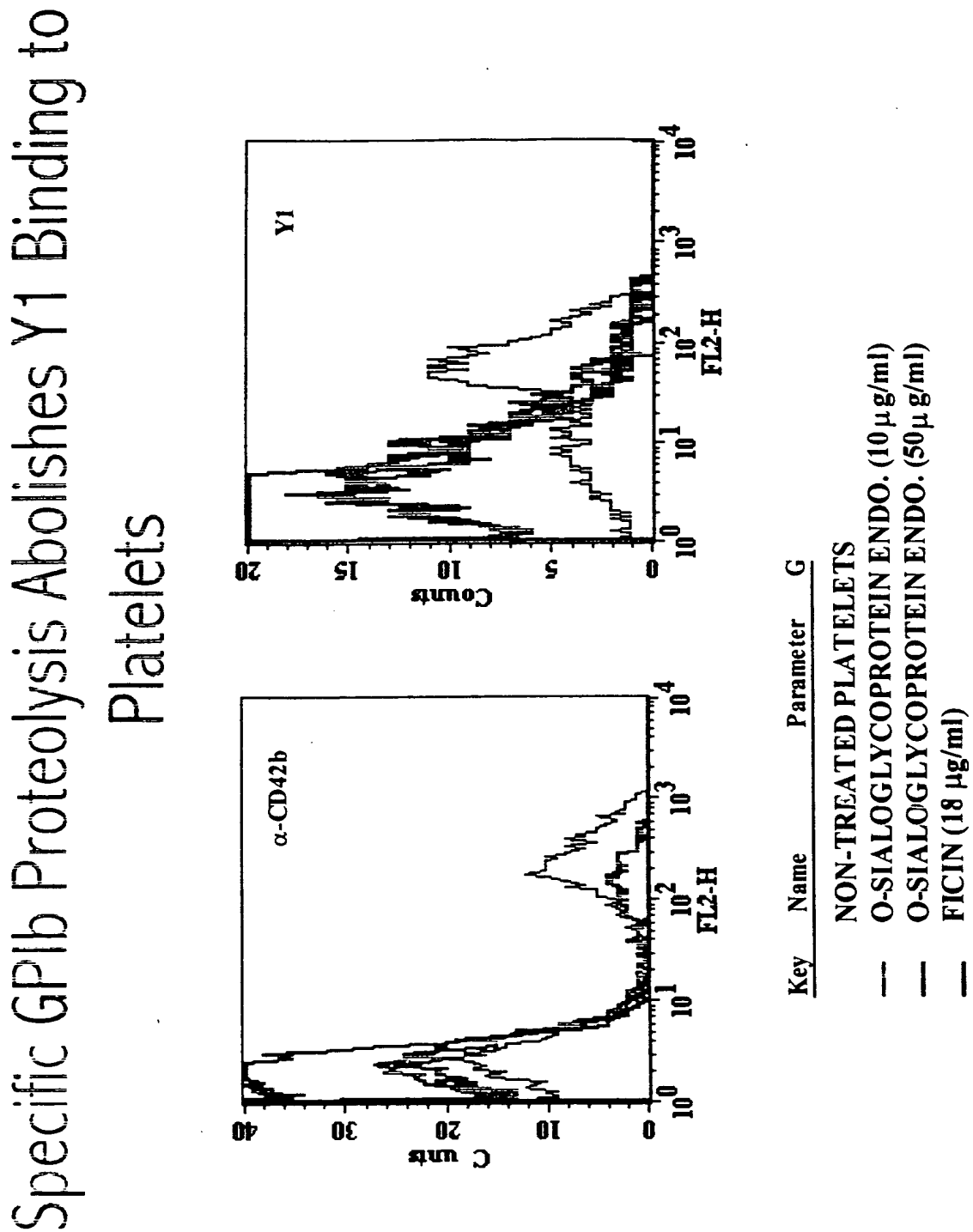


*FIG. 7*

Y1 binds N-terminal (His-1 - Glu 282) fragment of platelet GPIb after cleavage by mocarhagin

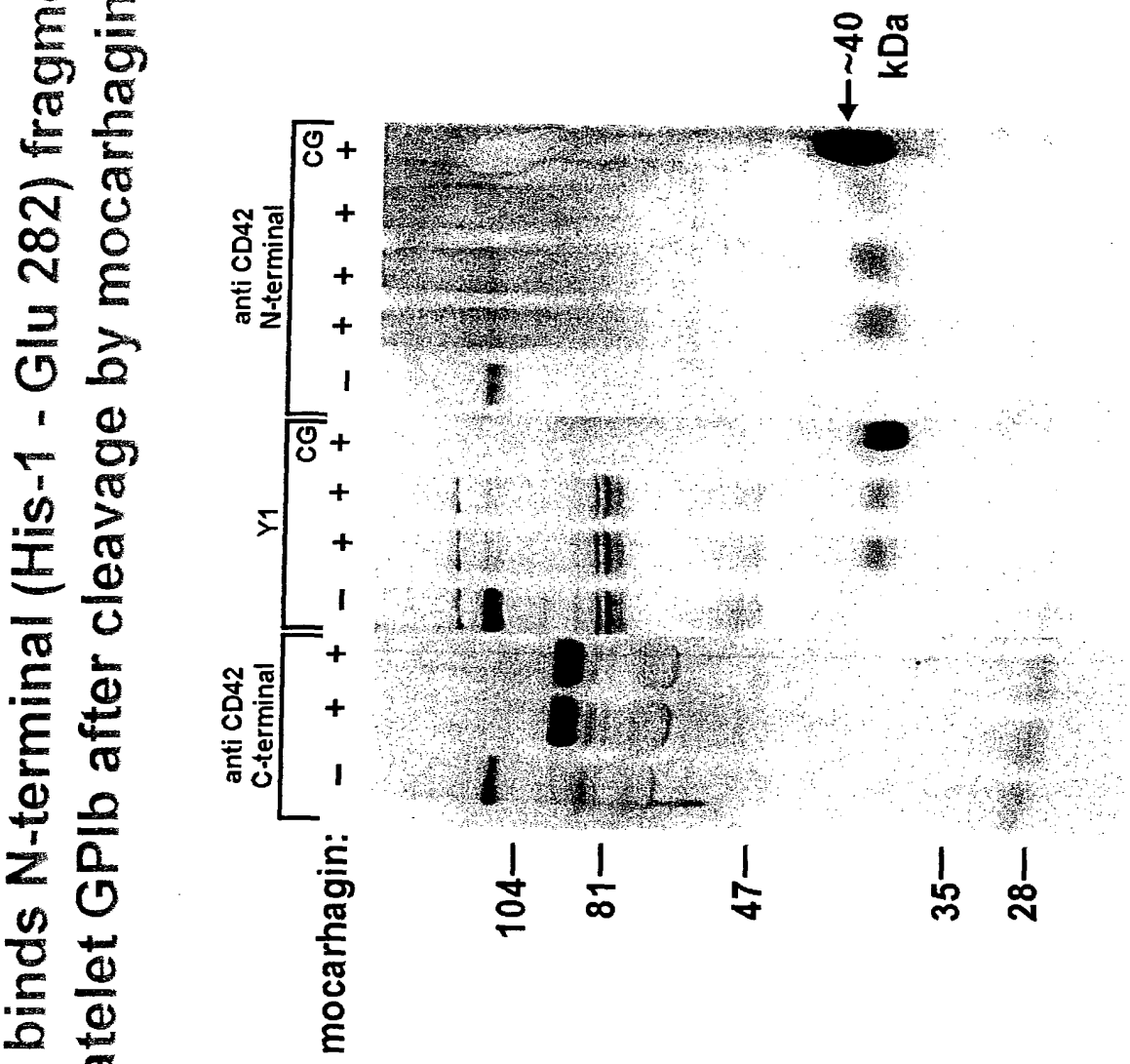


*FIG. 8*

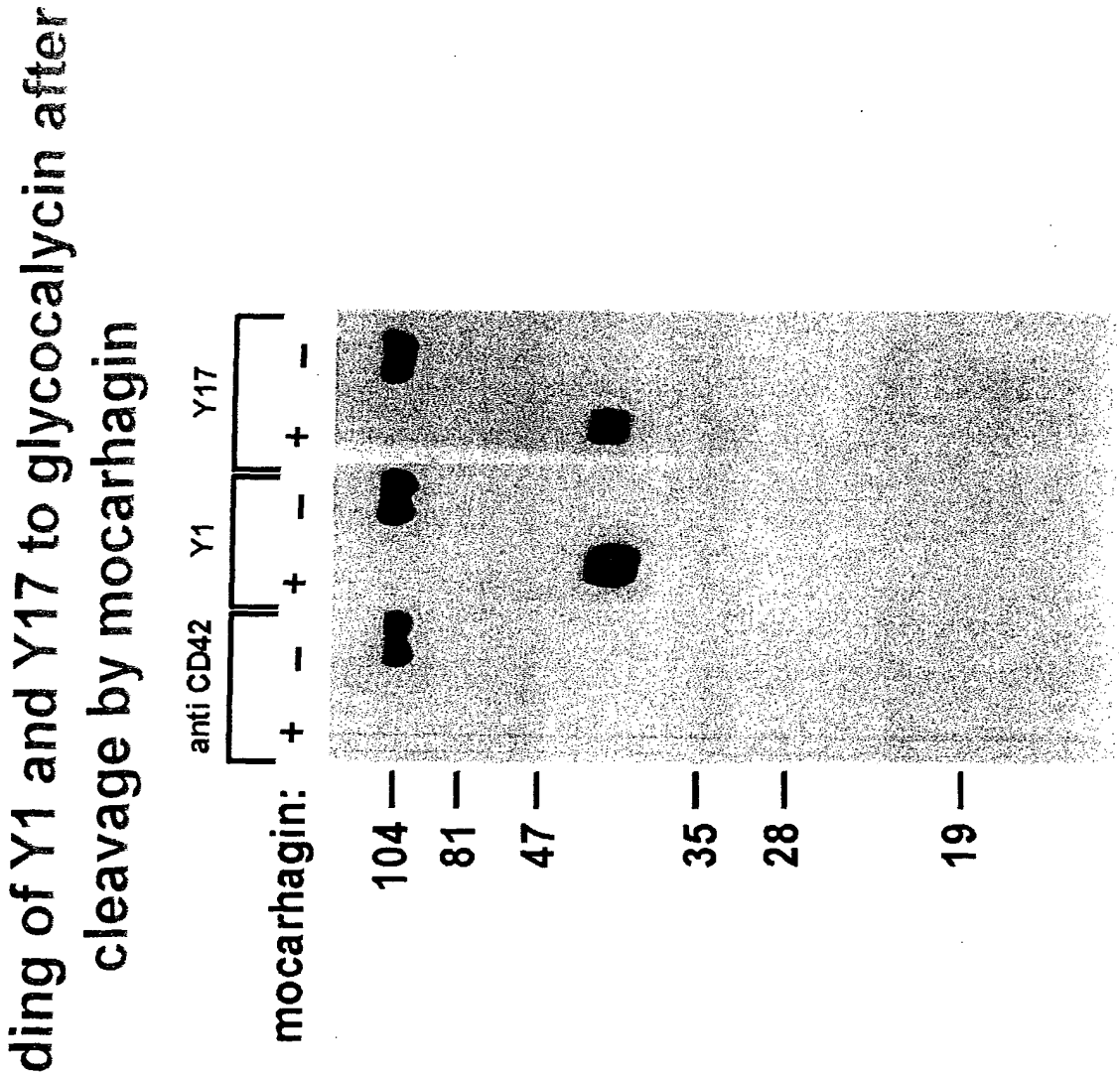


*FIG. 9*

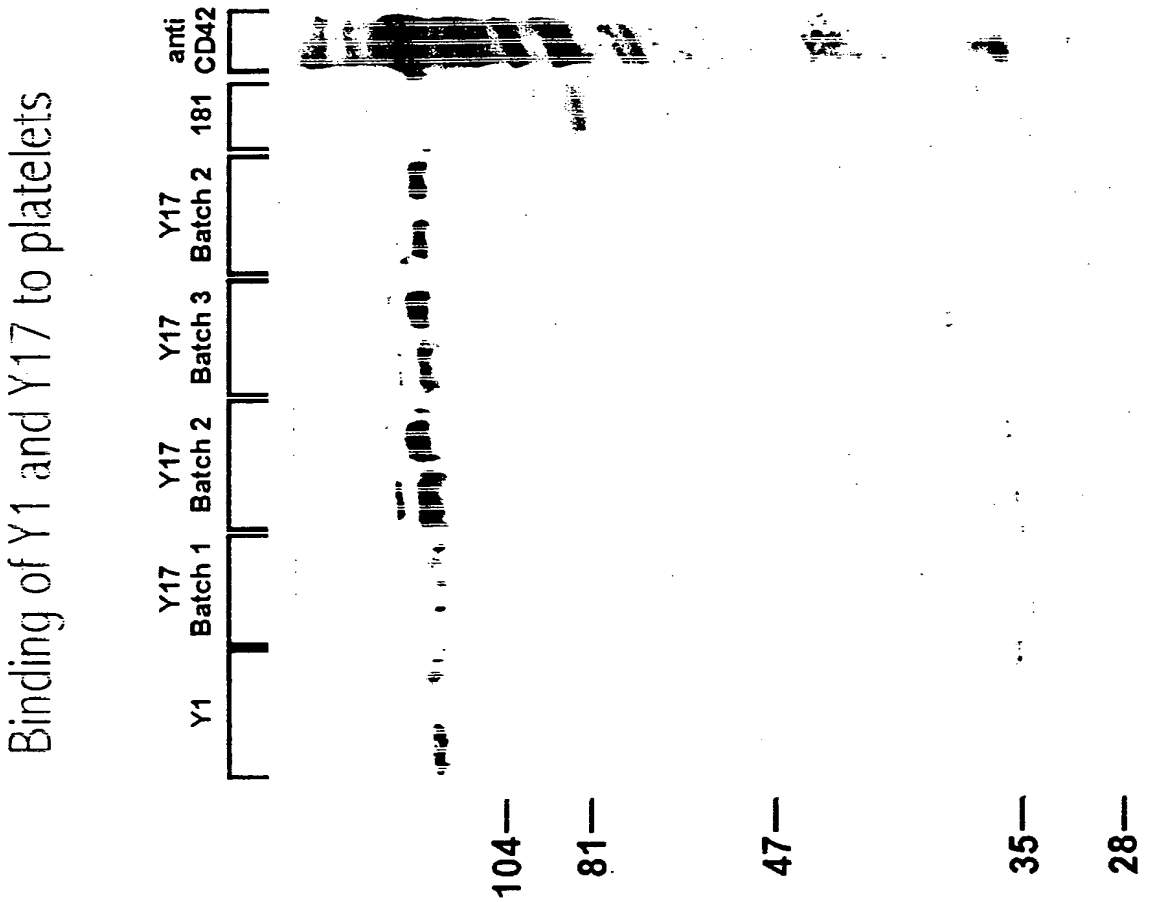


*FIG. 10*

Y1 and Y17 bind glycocalycin similar after cleavage by Ficin

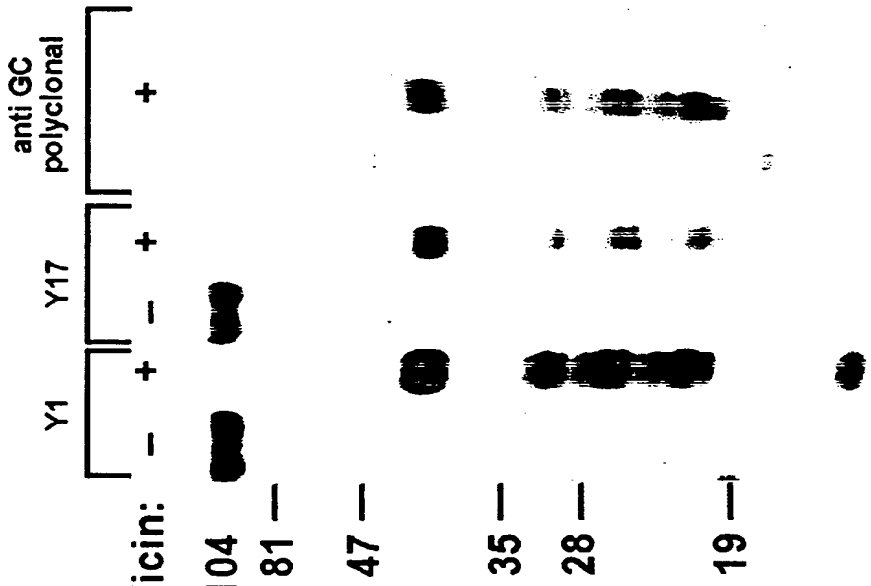


*FIG. 11*

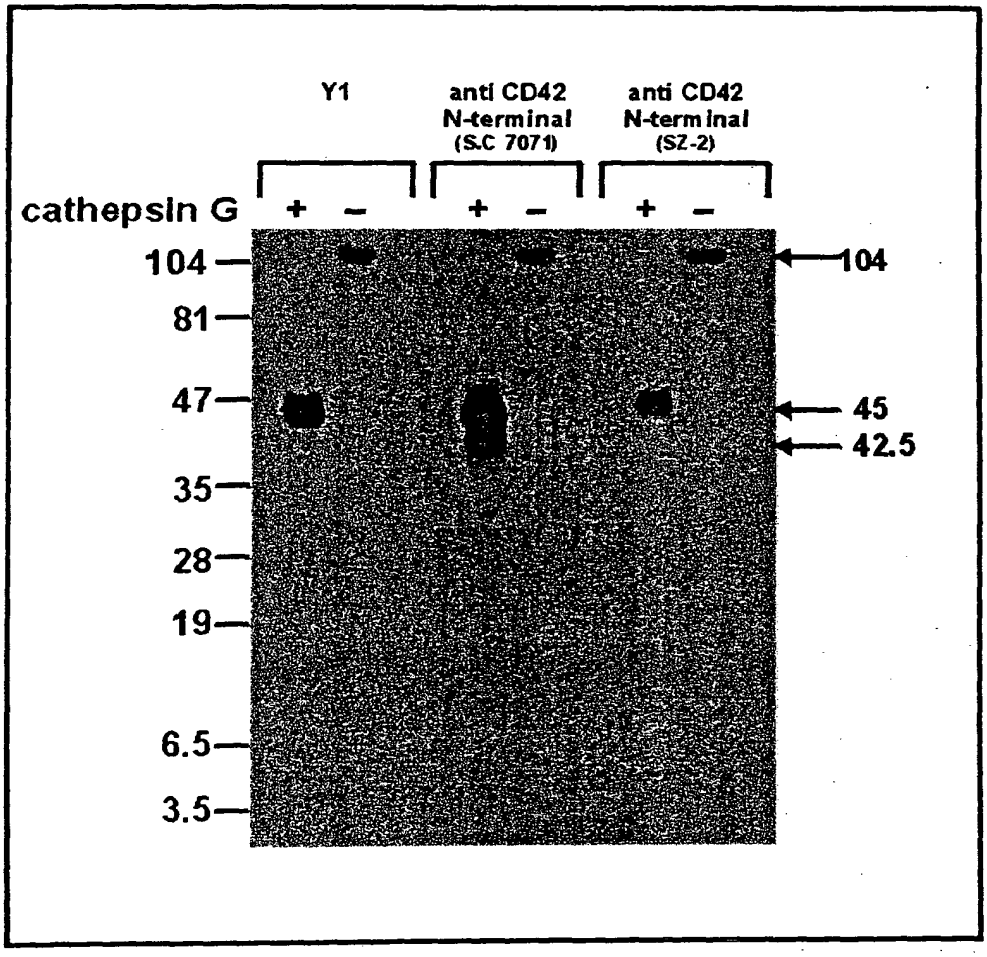


*FIG. 12*

Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIb fragment

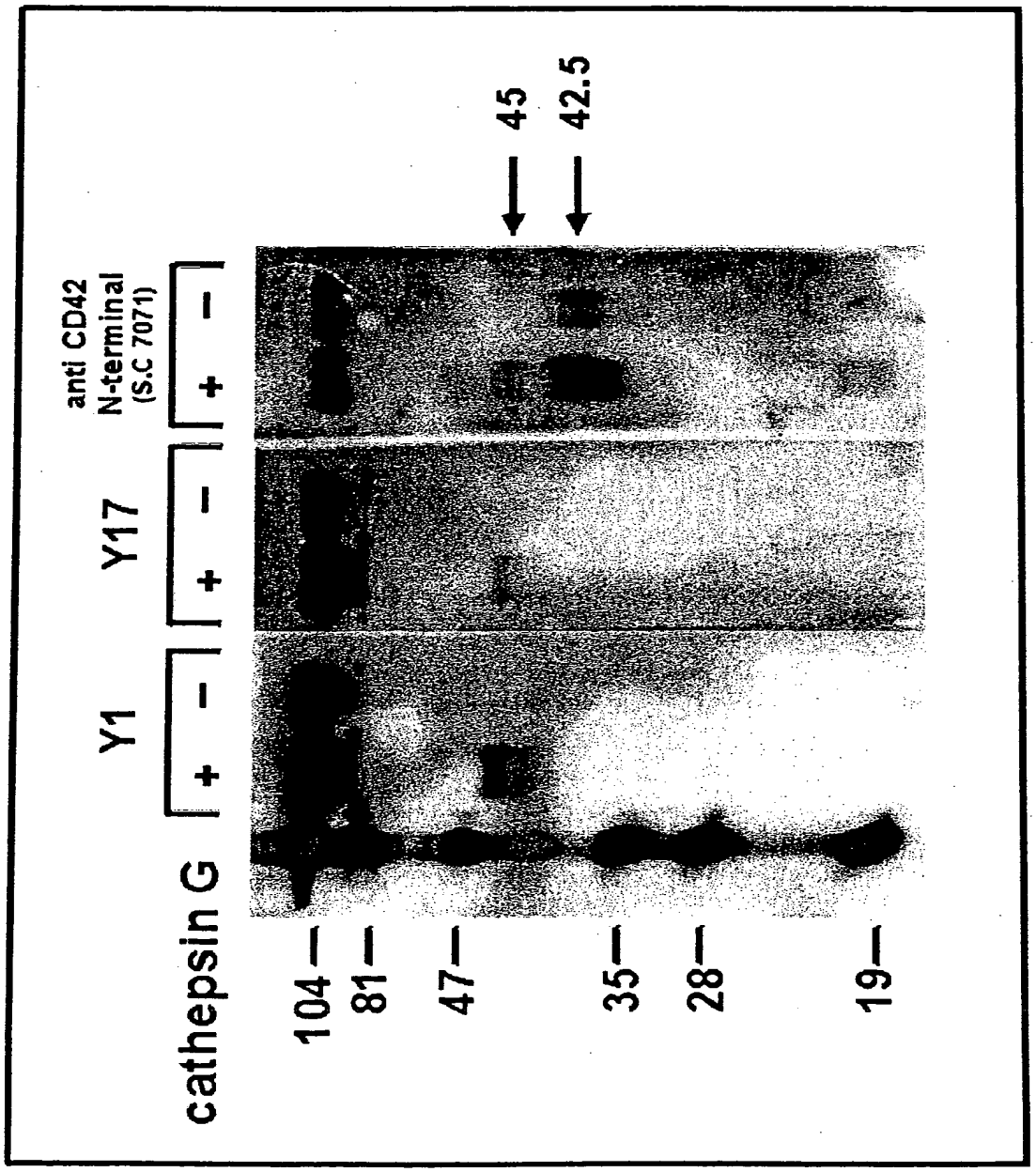


*FIG. 13*

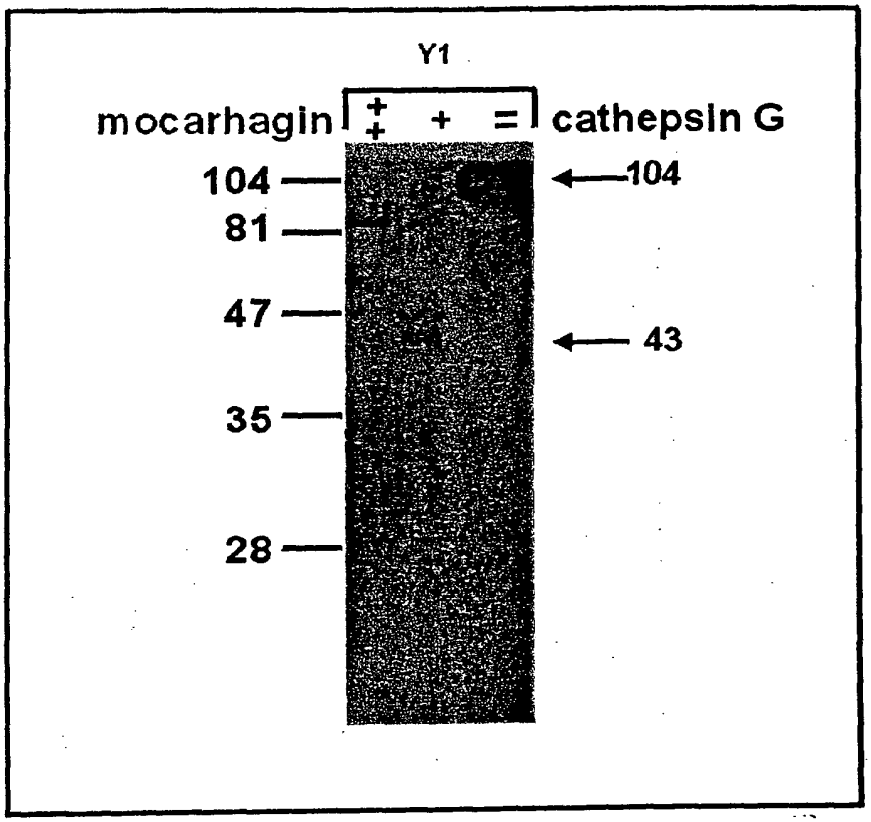


*FIG. 14*

Cleavage of washed platelets by mocarhagin and cathepsin G

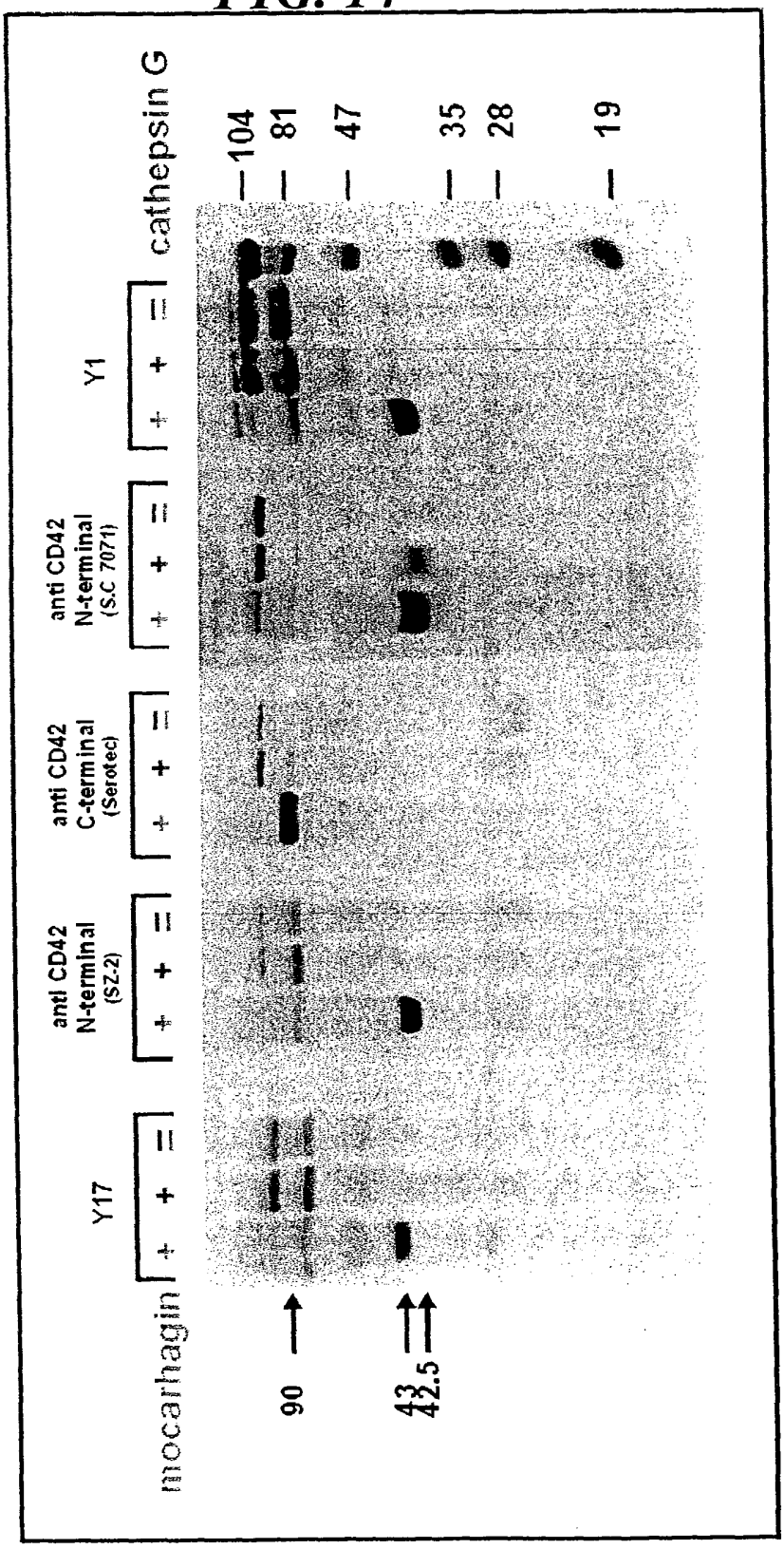


*FIG. 15*

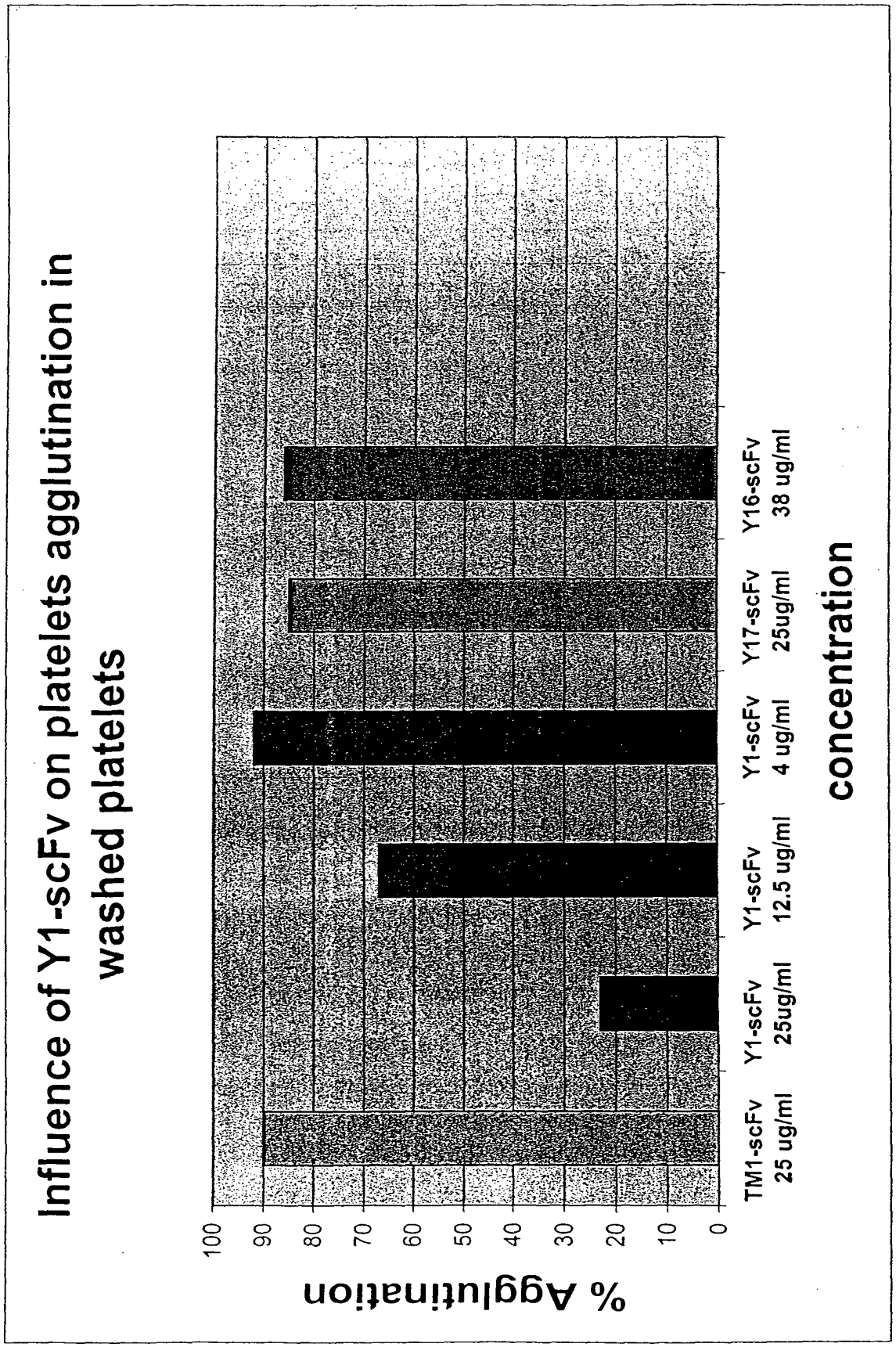


*FIG. 16*

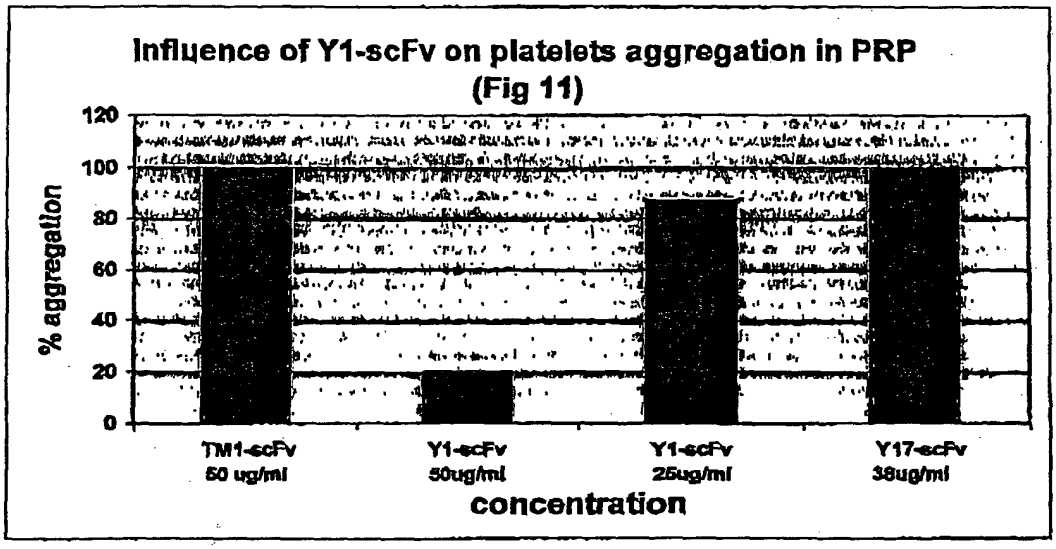


*FIG. 17*

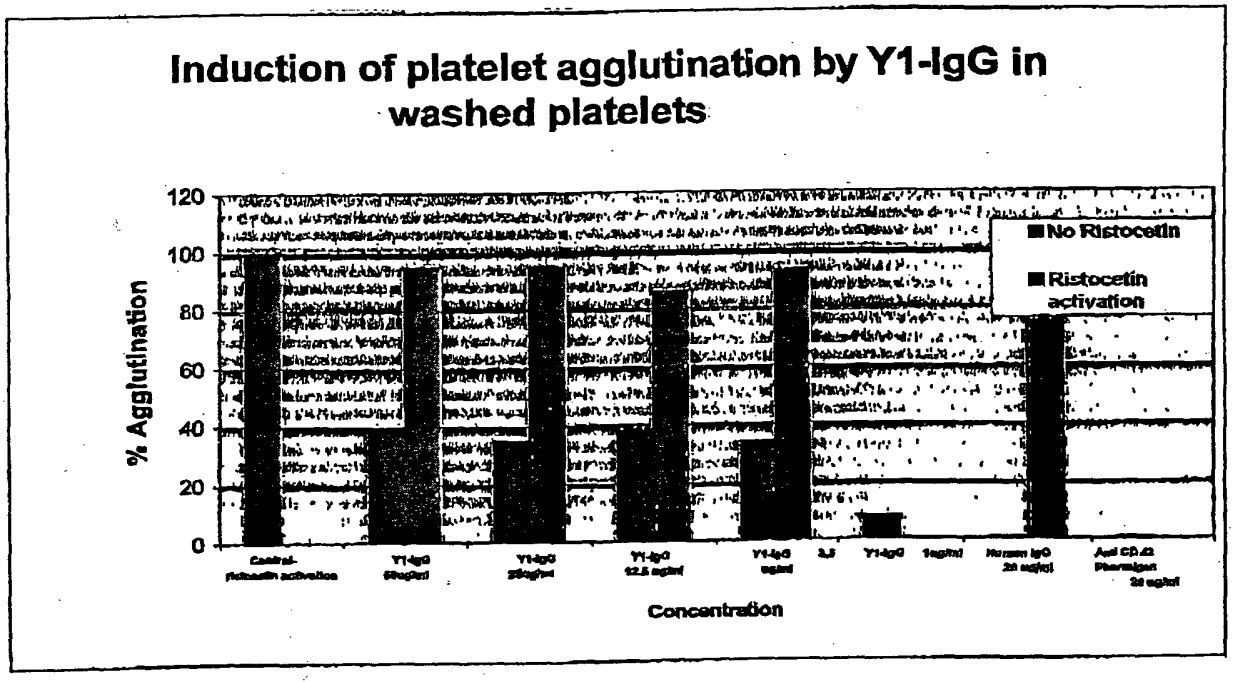
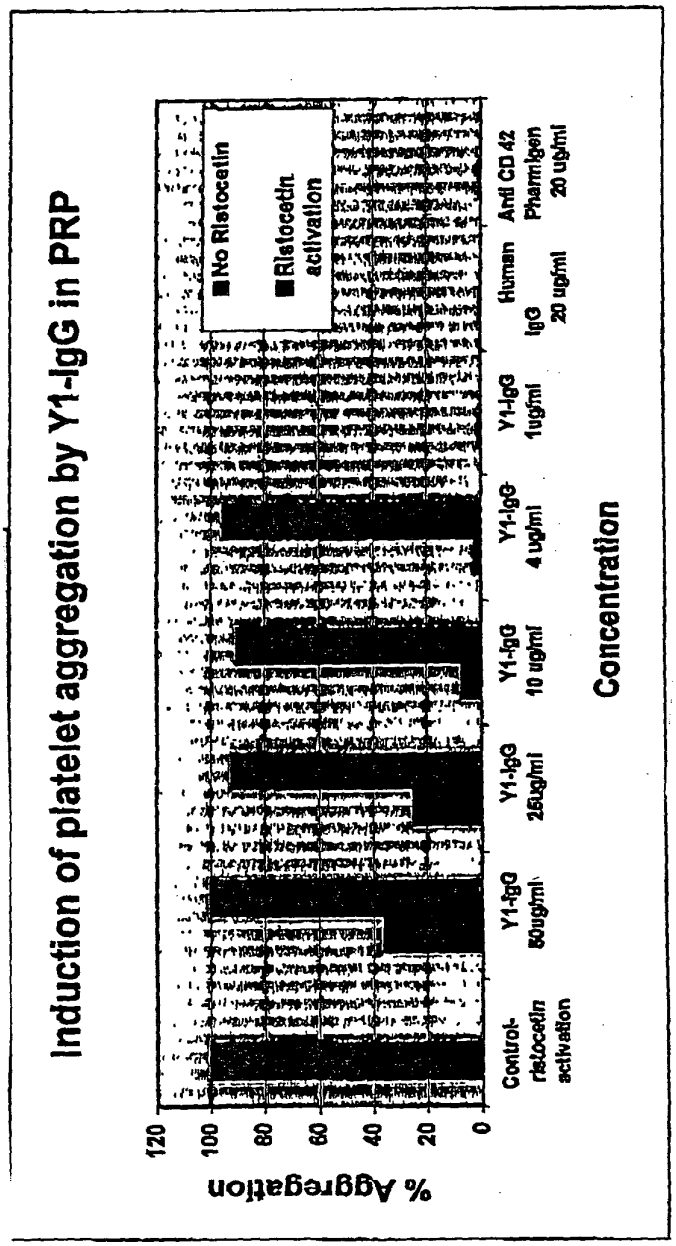


*FIG. 18*



# *FIG. 19*

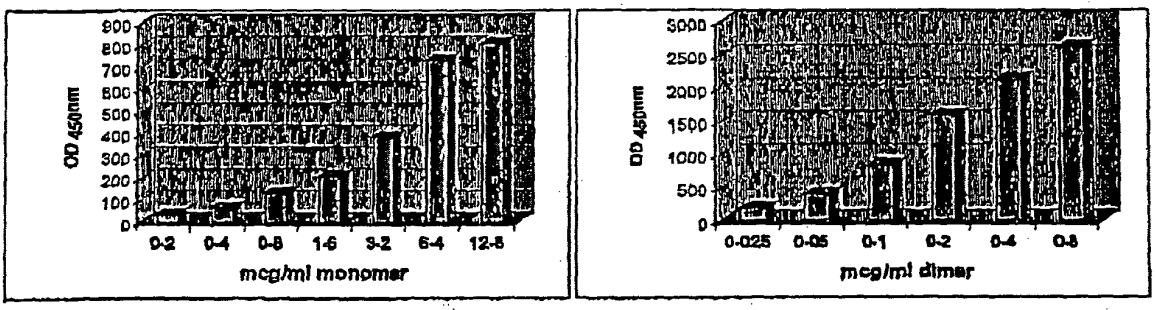
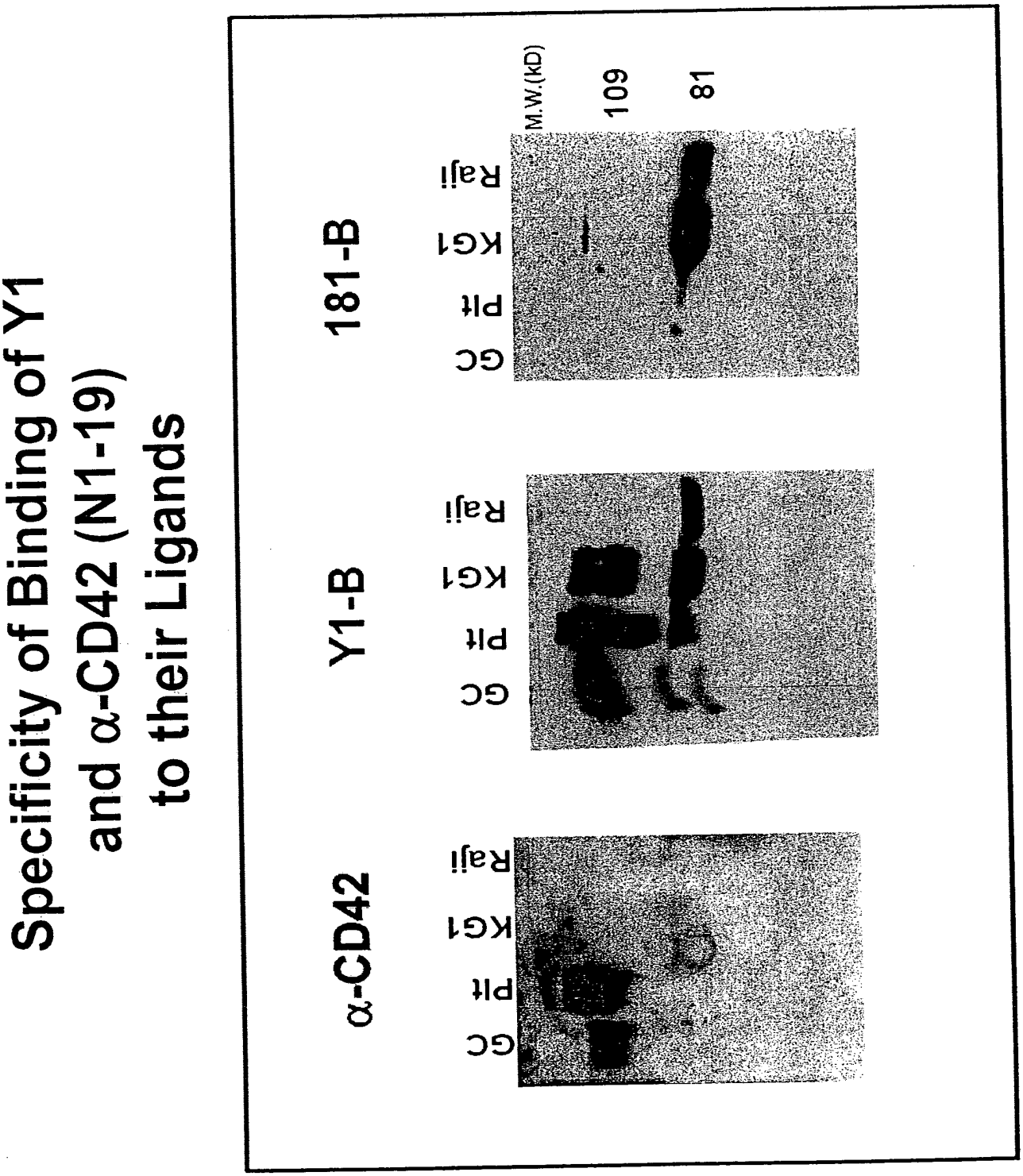


*FIG. 20*



# Y1-Ligand from KG1 membranes following Immuno-Precipitation with Y1: Purification on RP-HPLC

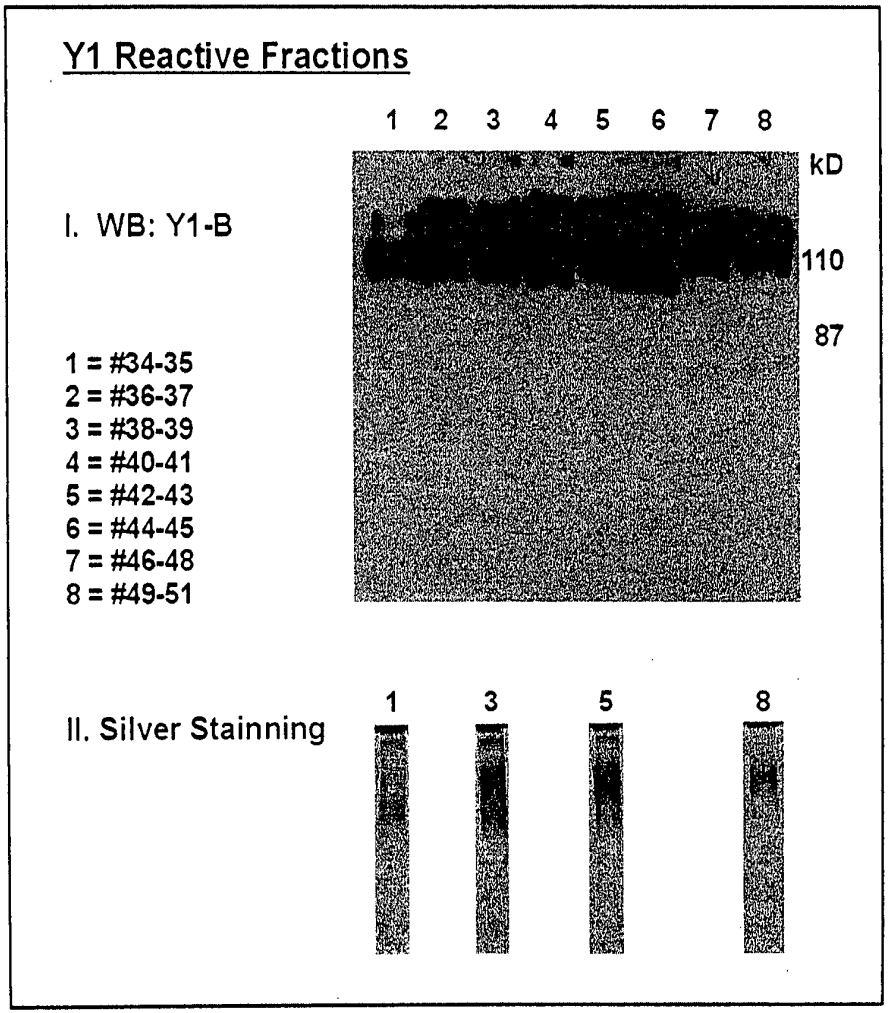



FIG. 21

*FIG. 22*

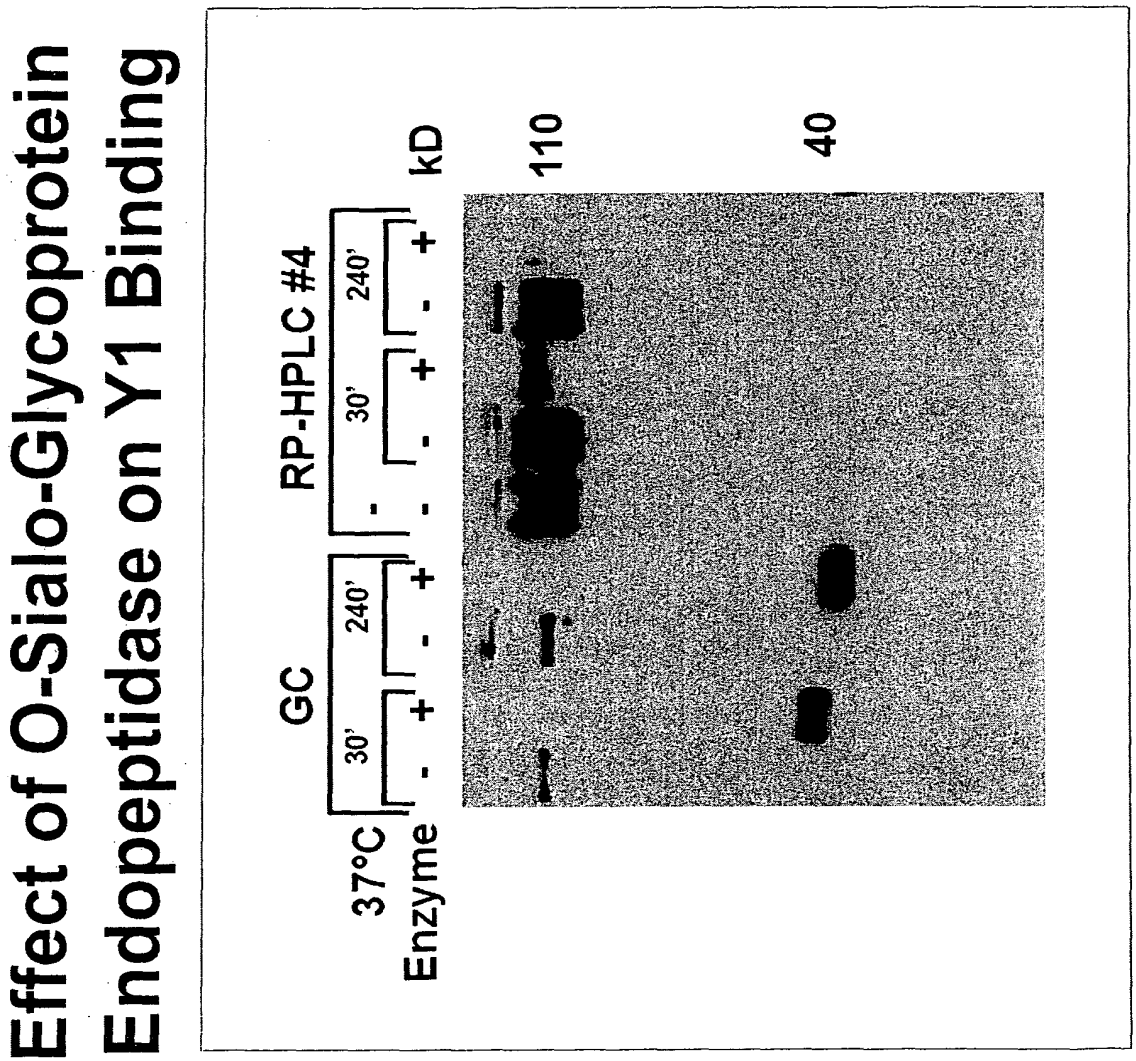
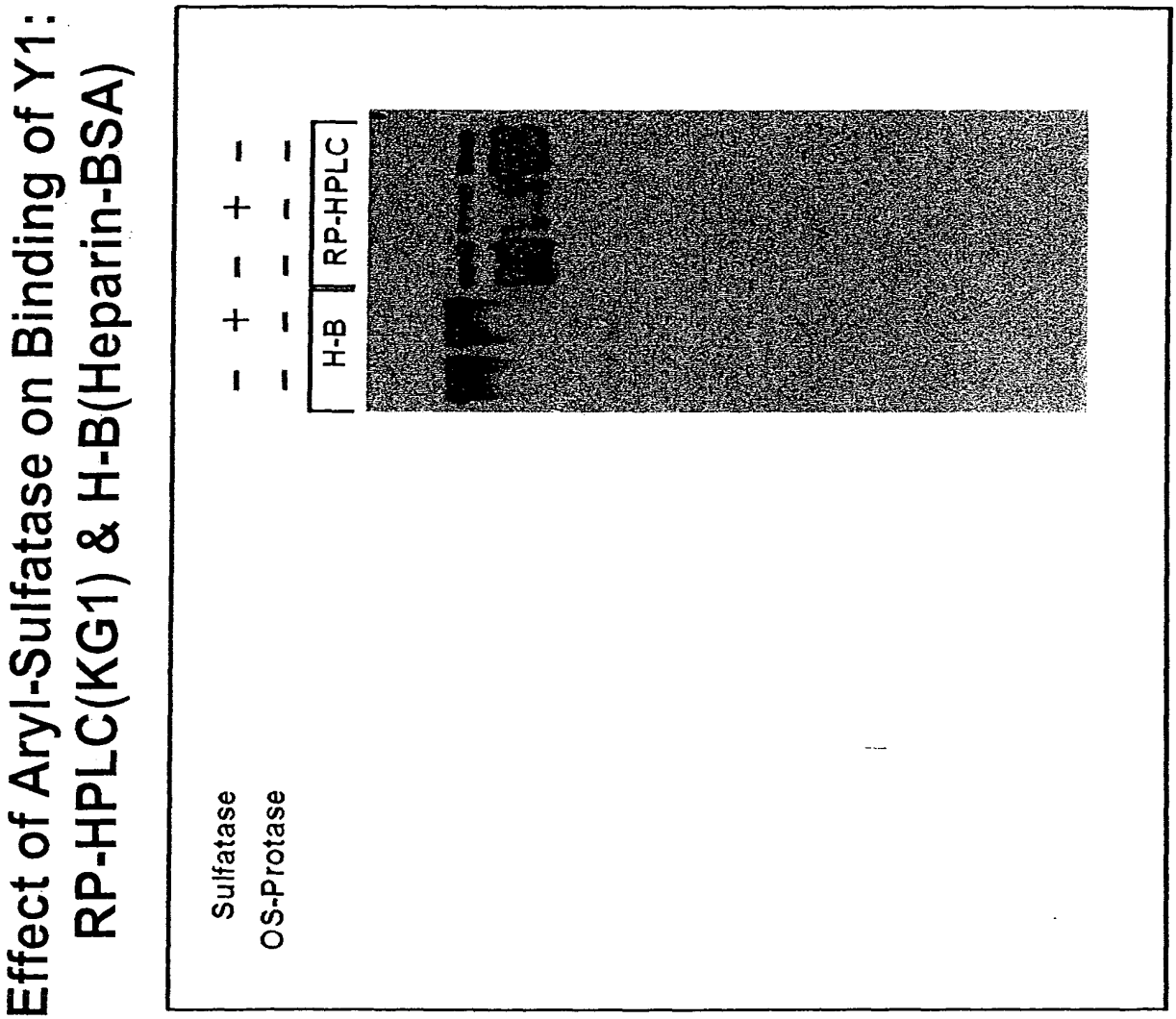
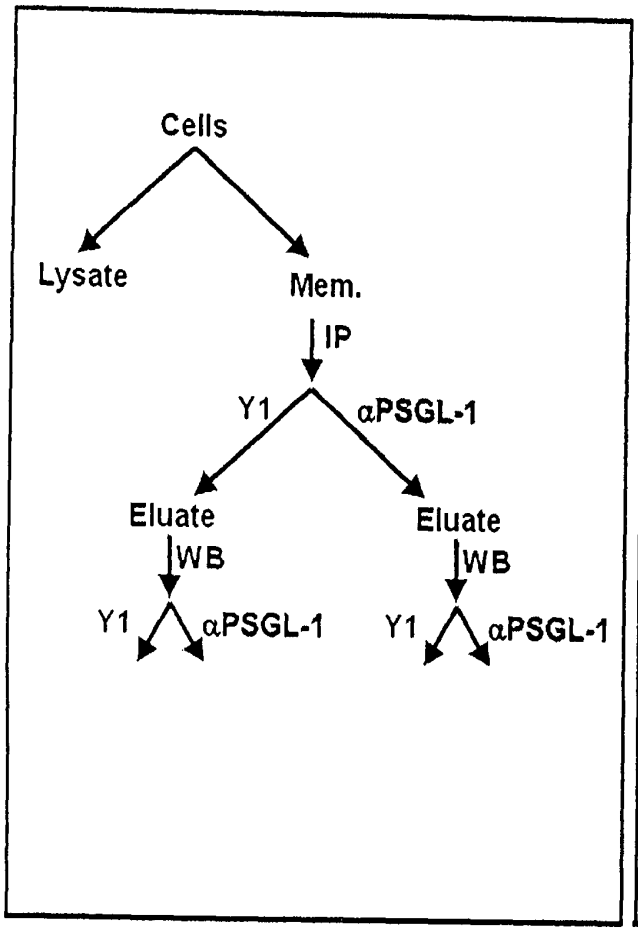


*FIG. 23*



# Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1



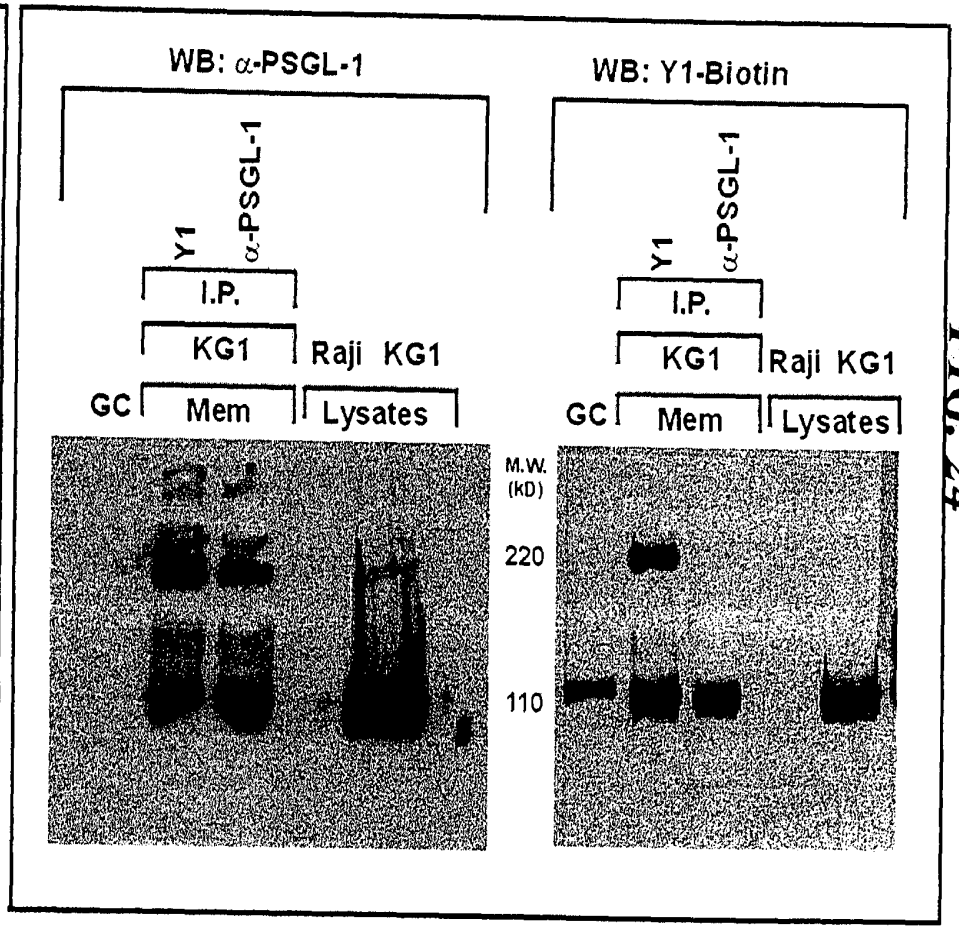
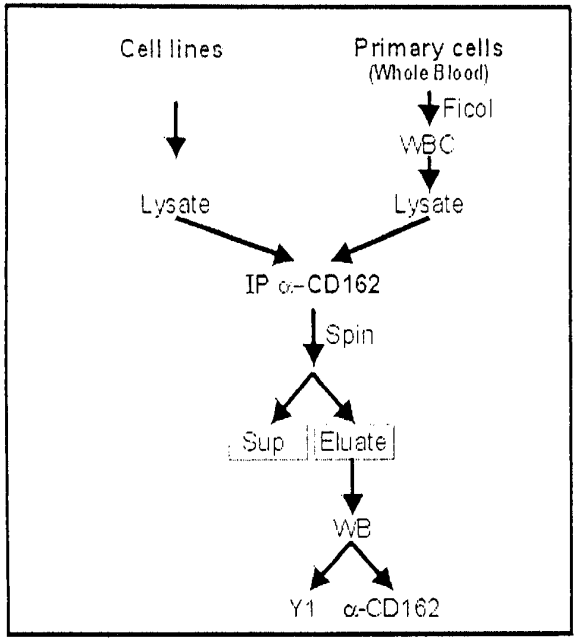
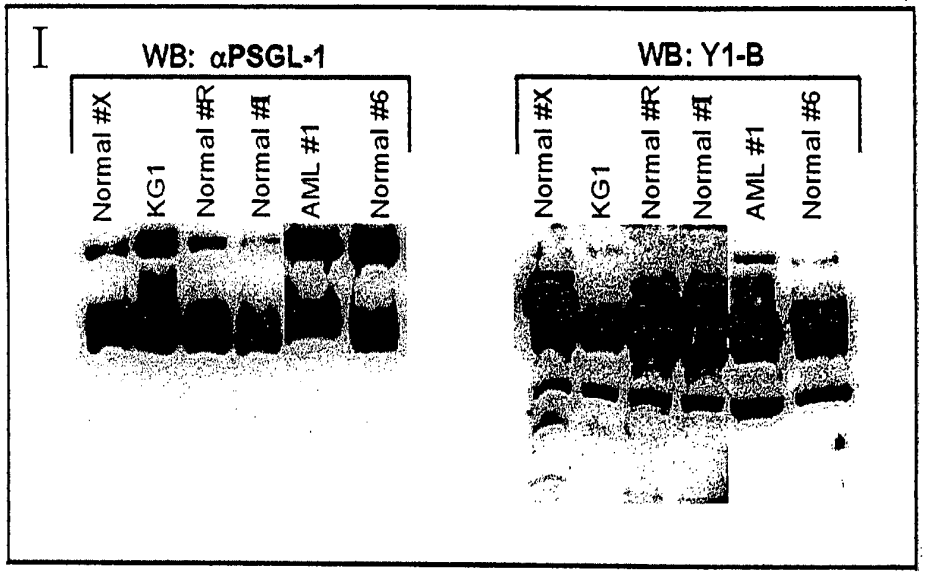




FIG. 24

α-CD162 and Y1:
Comparison between cells from AML patient and normal blood



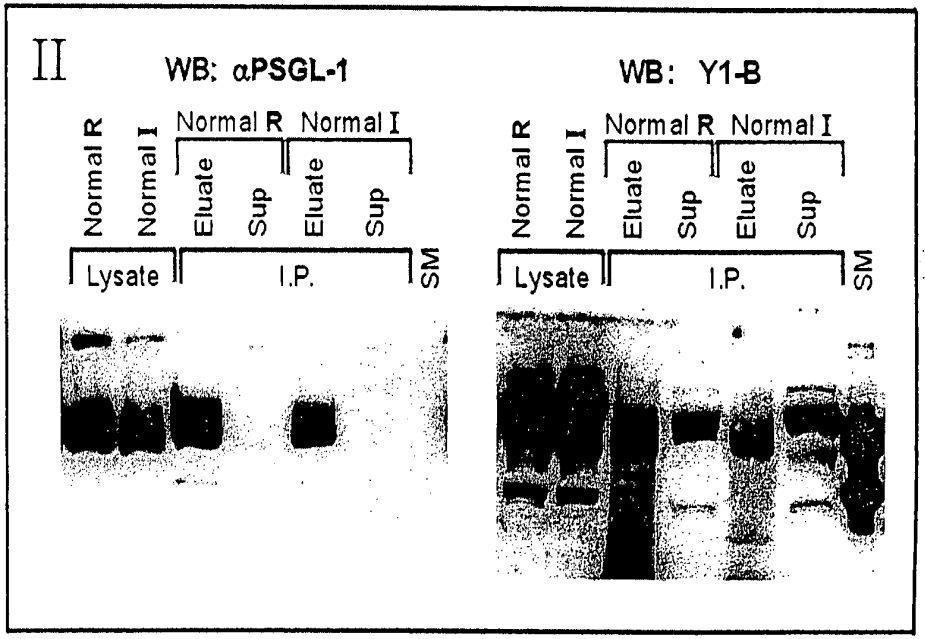
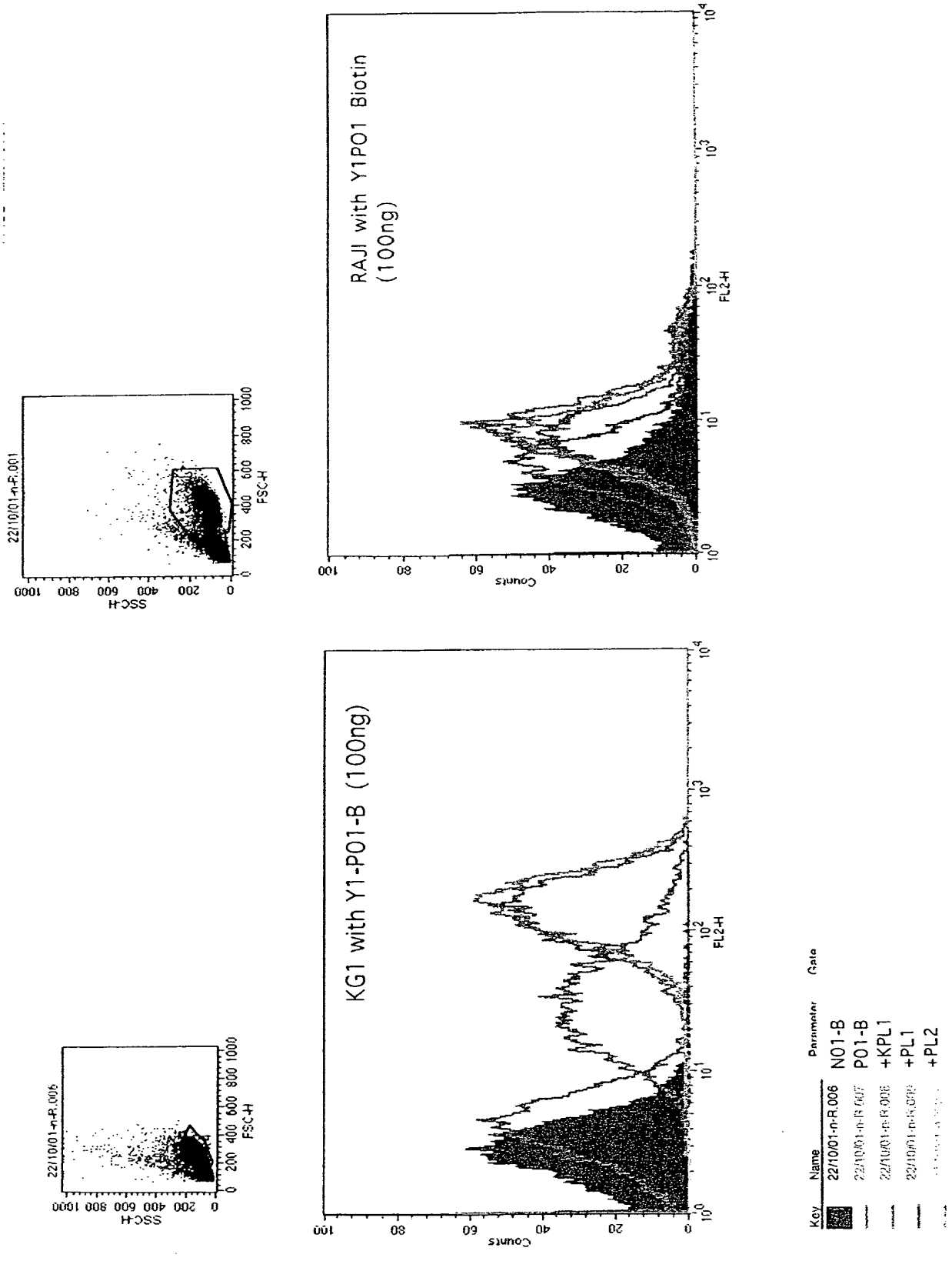
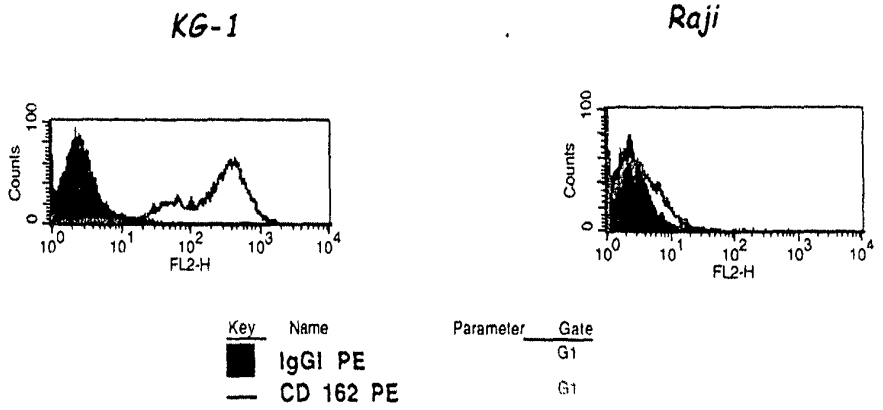




FIG. 25

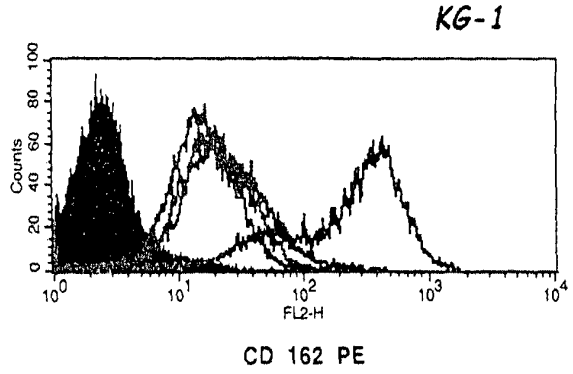
# FIG. 26



# Specificity of Y1 Binding: Analysis by FACS




## Binding of α PSGL1 (αCD162/KPL1); competition with Y1-IgG



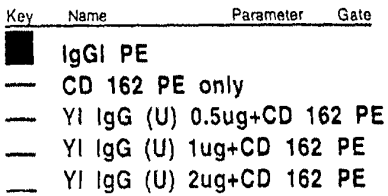
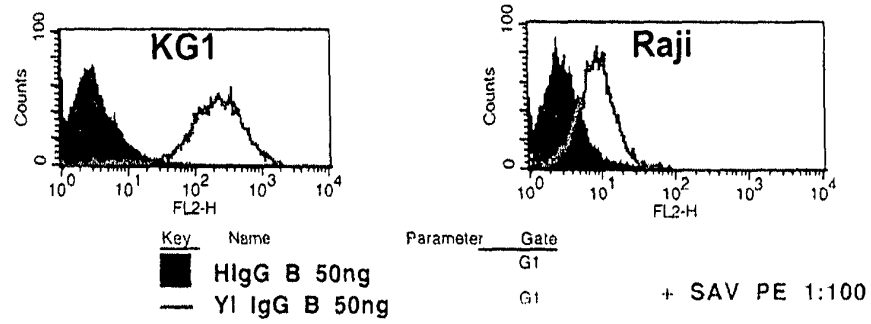




FIG. 27

# Specificity of Y1 Binding: Analysis by FACS




- Binding of Y1-IgG; competition with αPSGL-1 (CD162 /KPL1)

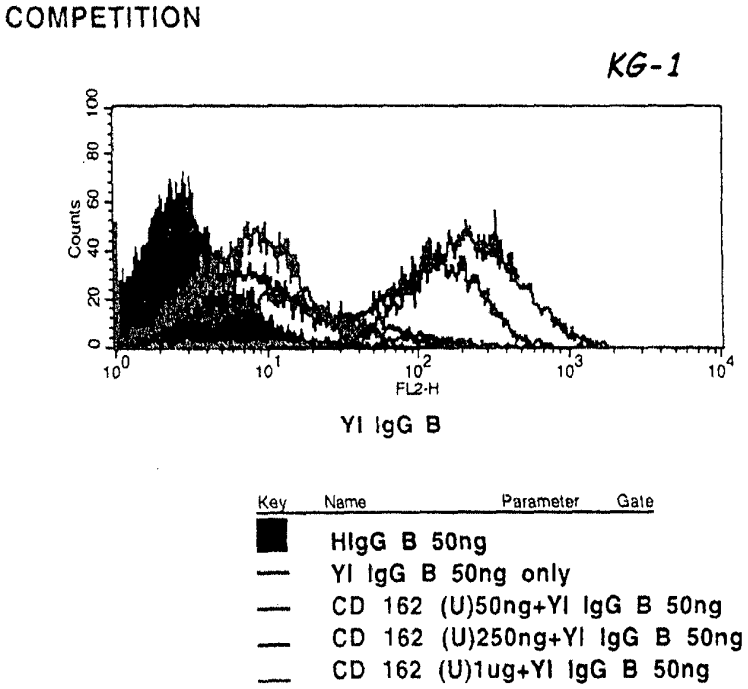
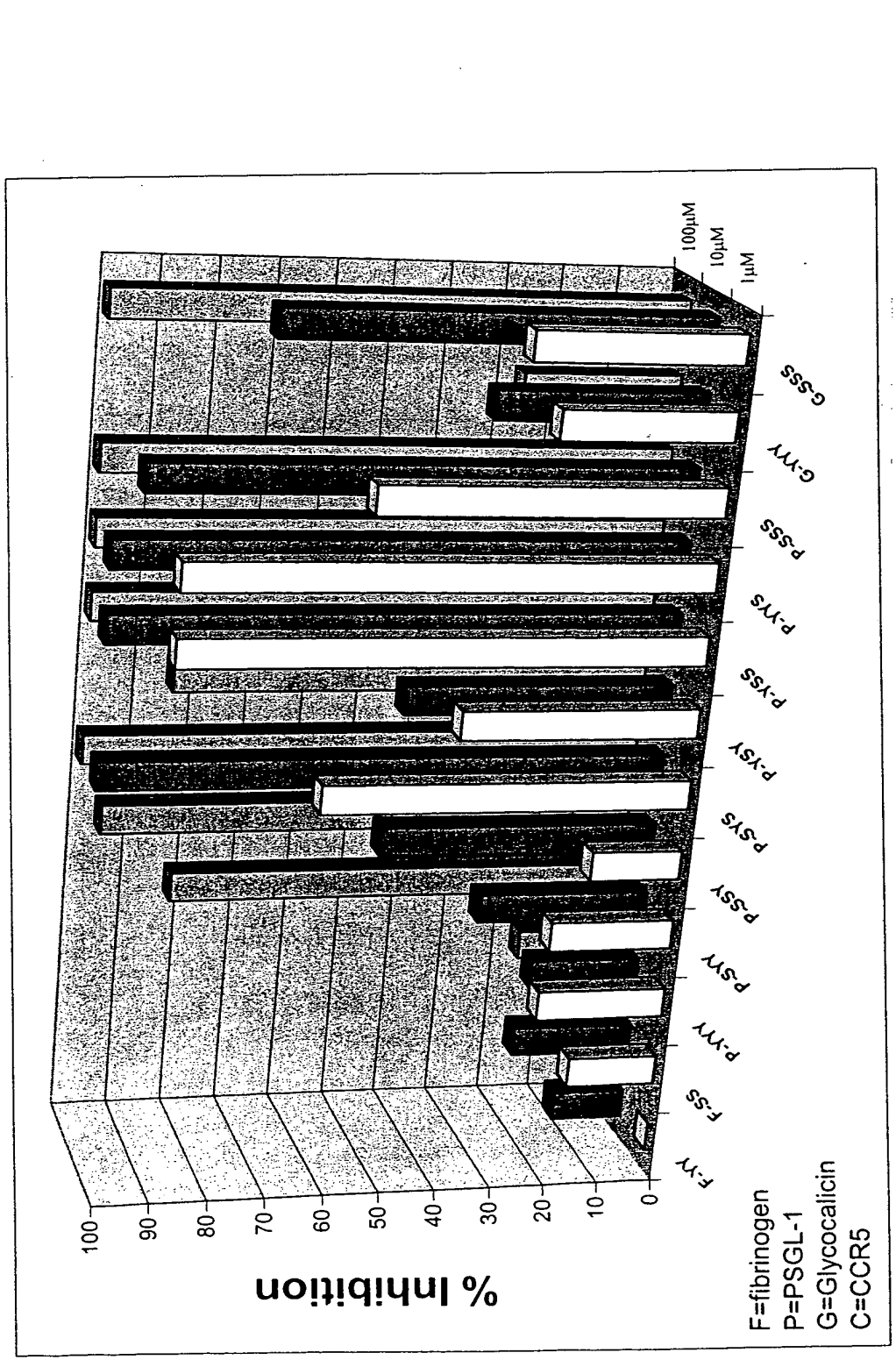


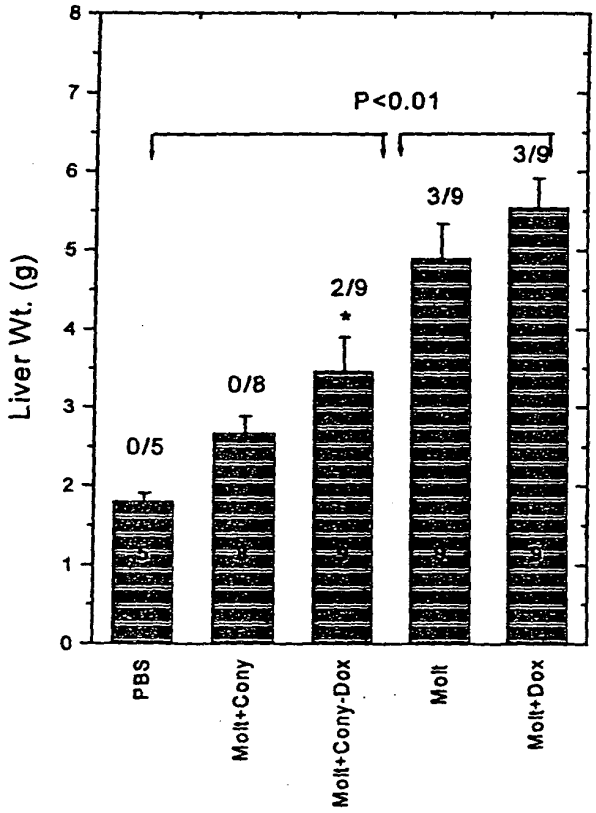

FIG. 28

# FIG. 29



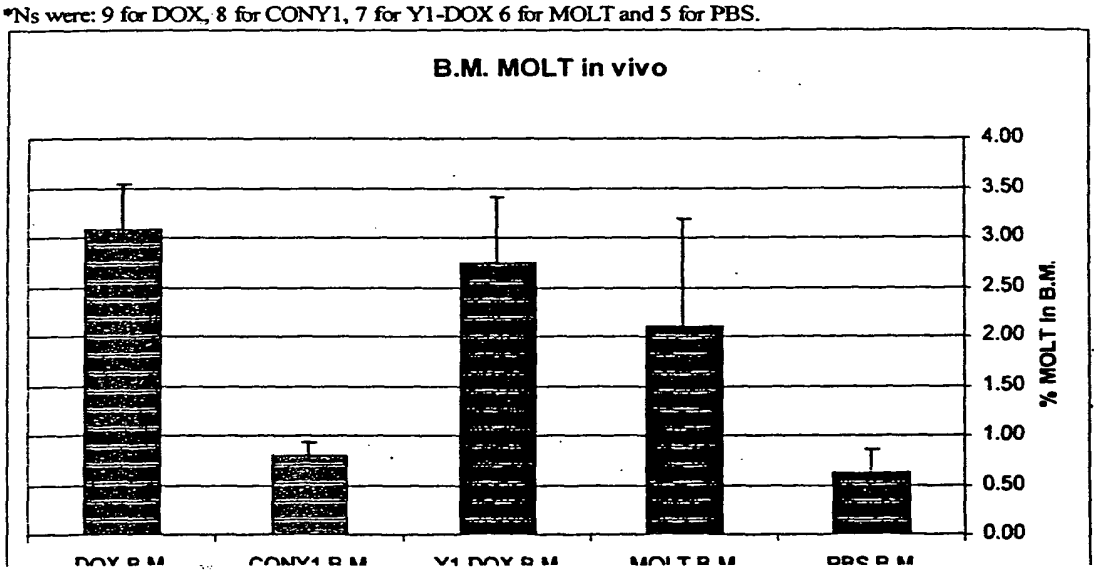

| | F-YY | F-SS | P-YYY | P-SYY | P-SSY | P-SYS | P-YSY | P-YSS | P-YYS | P-SSS | G-YYY | G-SSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100μM | 0 | 0 | 0 | 18 | 83 | 96 | 100 | 85 | 100 | 100 | 100 | 28 | 100 |
| 10μM | 13 | 22 | 20 | 31 | 50 | 100 | 48 | 100 | 100 | 95 | 37 | 75 |
| 1μM | 0 | 16 | 23 | 22 | 16 | 65 | 42 | 91 | 91 | 60 | 30 | 36 |

*FIG. 30*

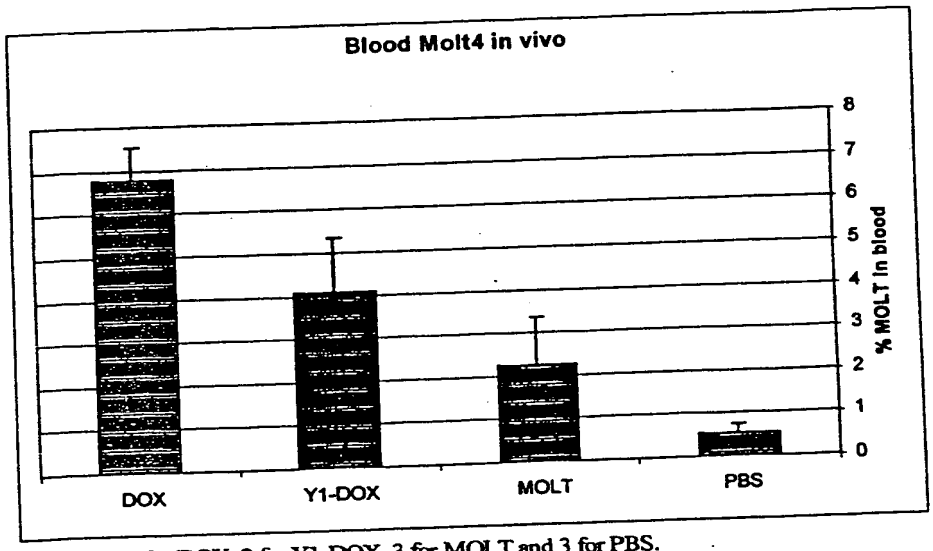


# *FIG. 31*

*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.



# *FIG. 32*




**Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.

*FIG. 33*

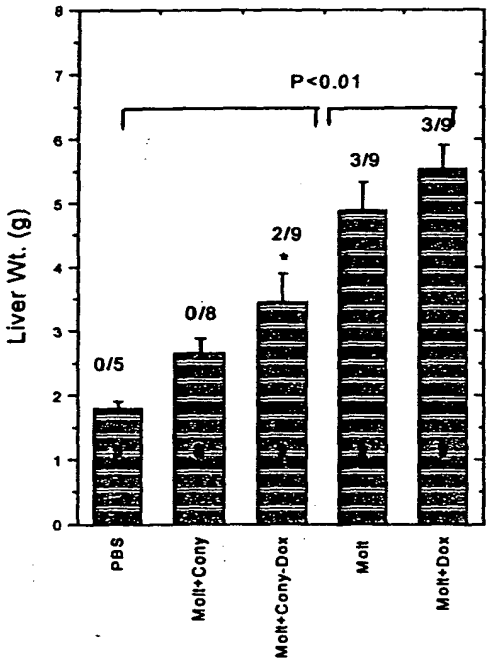
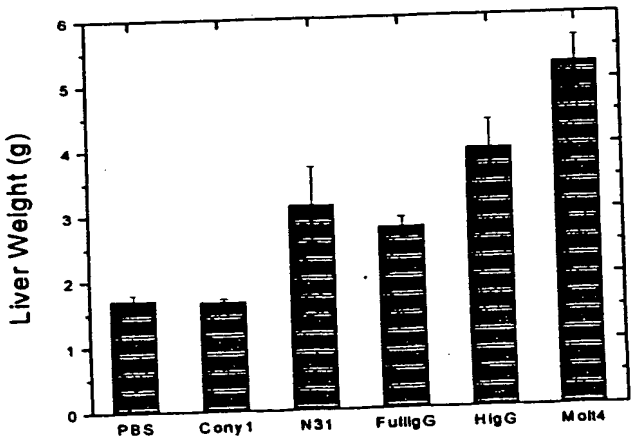
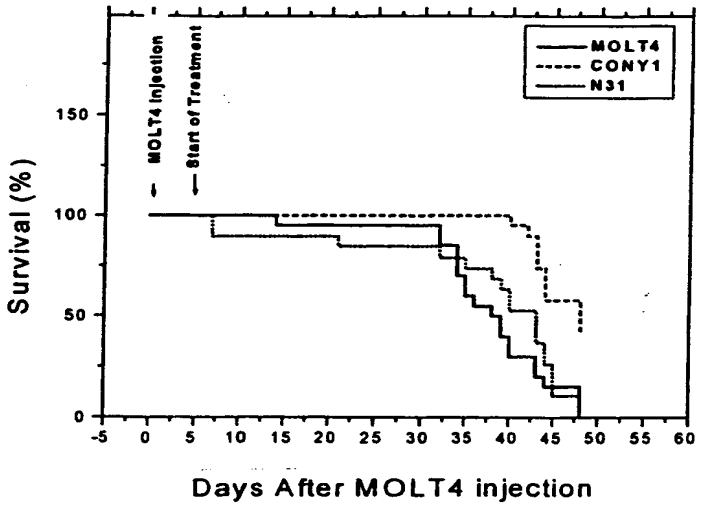


*FIG. 34*

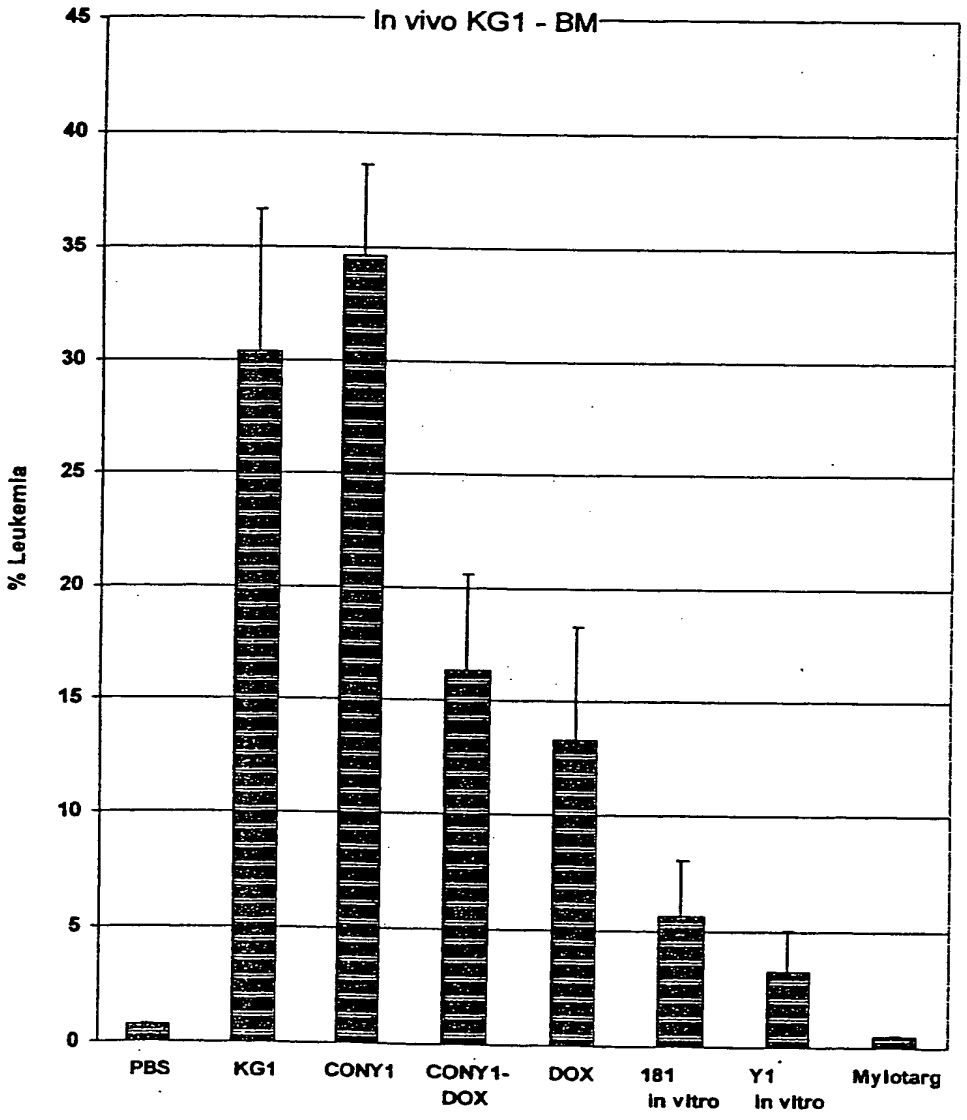


# *FIG. 35*

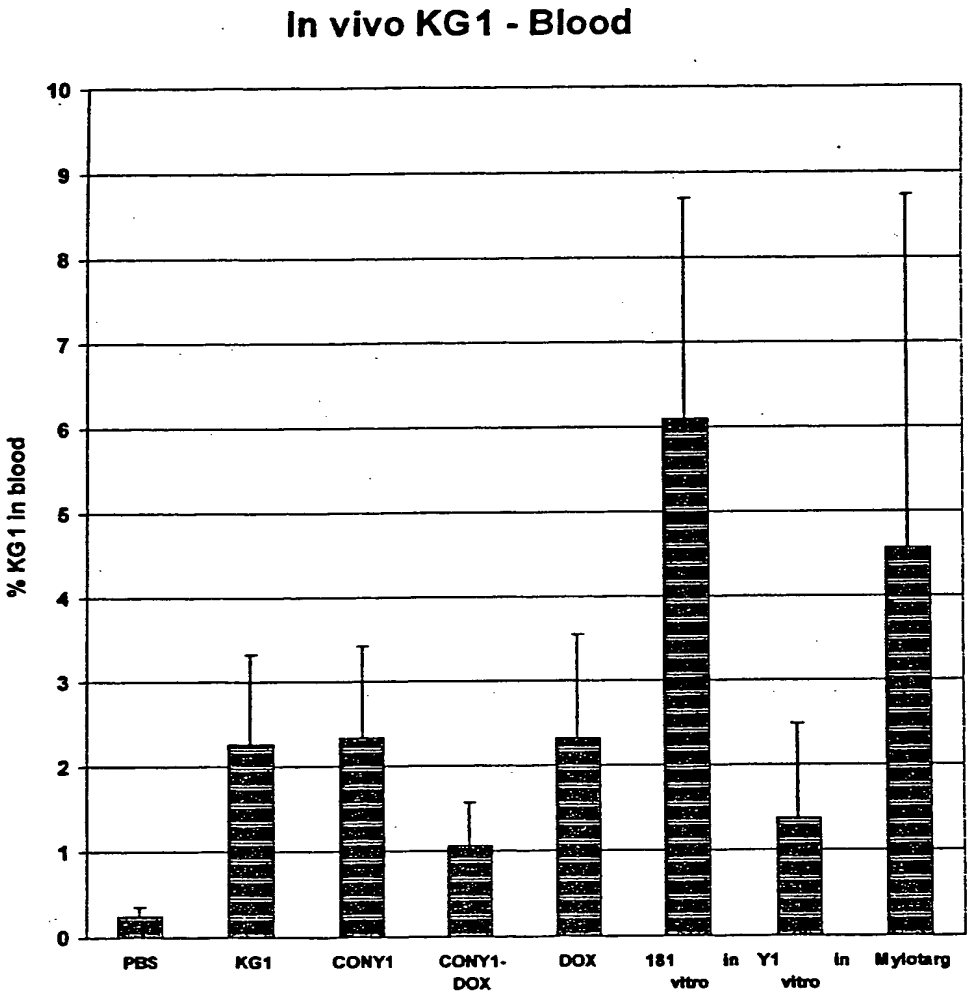


# FIG. 36

***Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.



# *FIG. 37*




****Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

*FIG. 38*

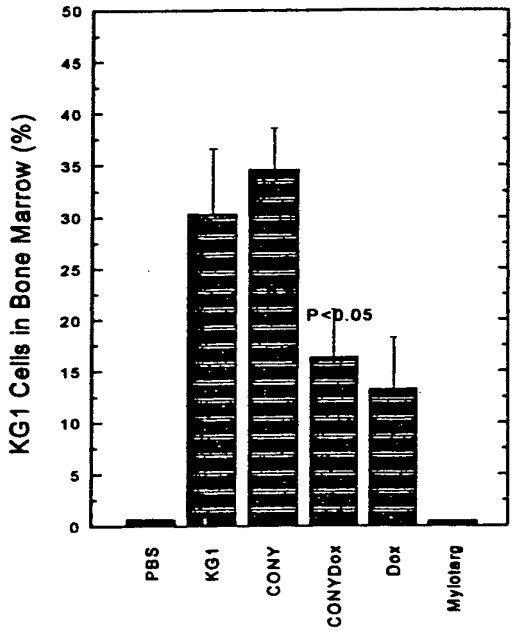


*FIG. 39*

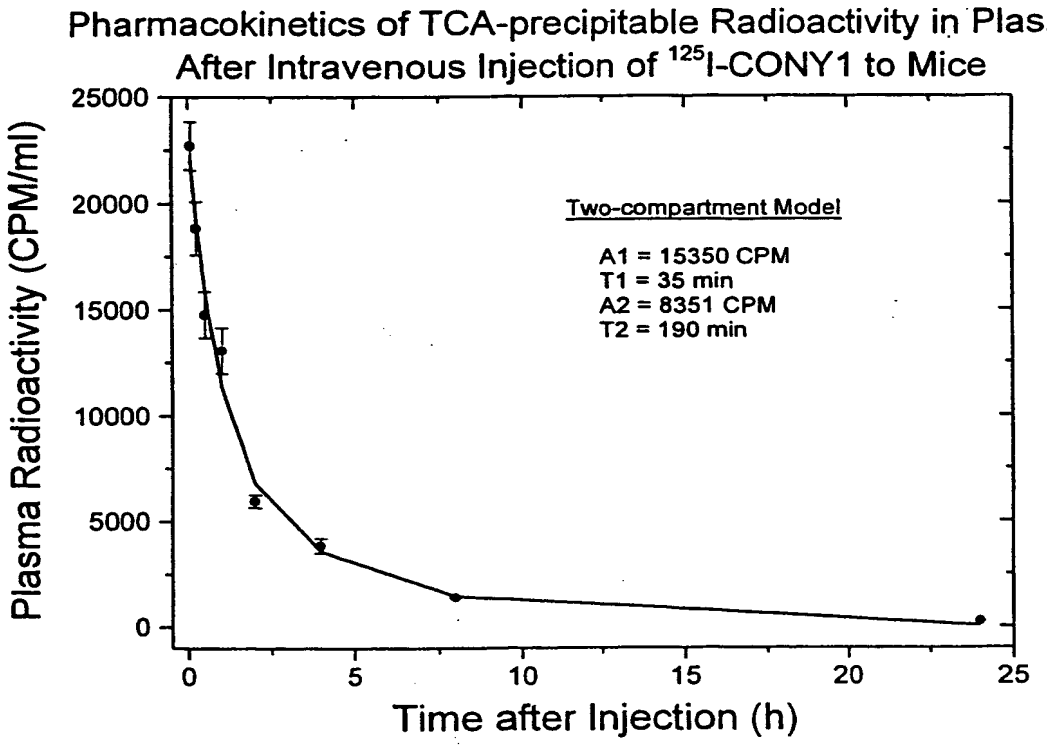


*FIG. 40*

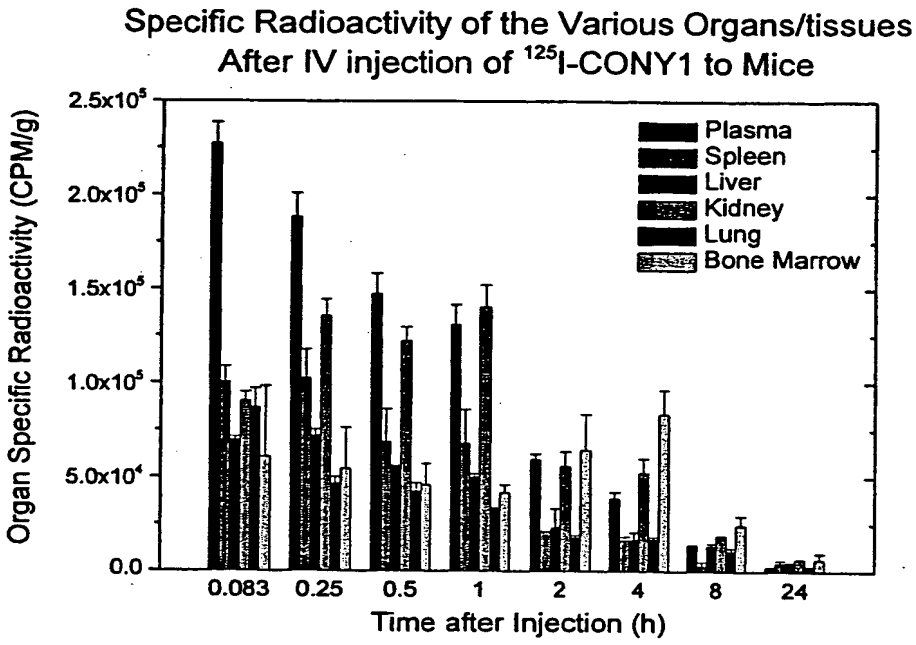


*FIG. 41*

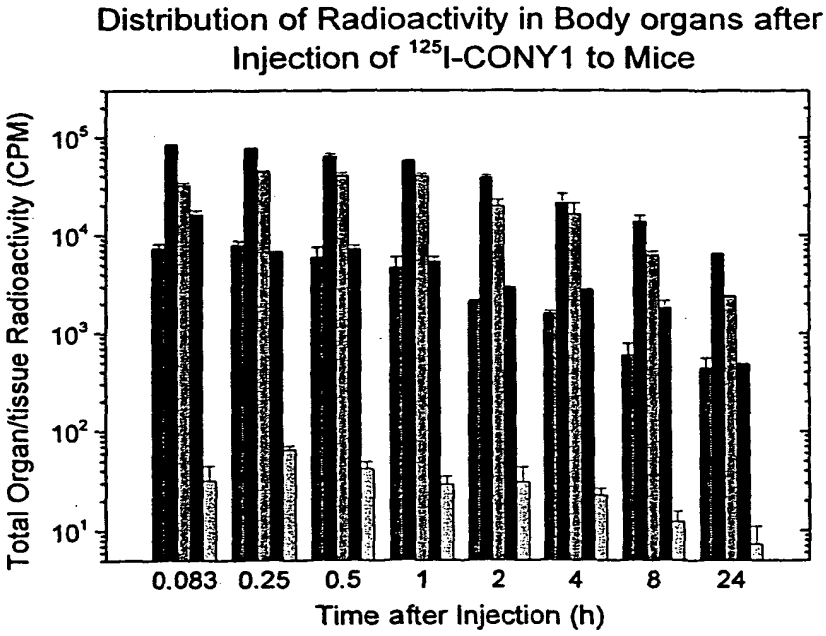


*FIG. 42*

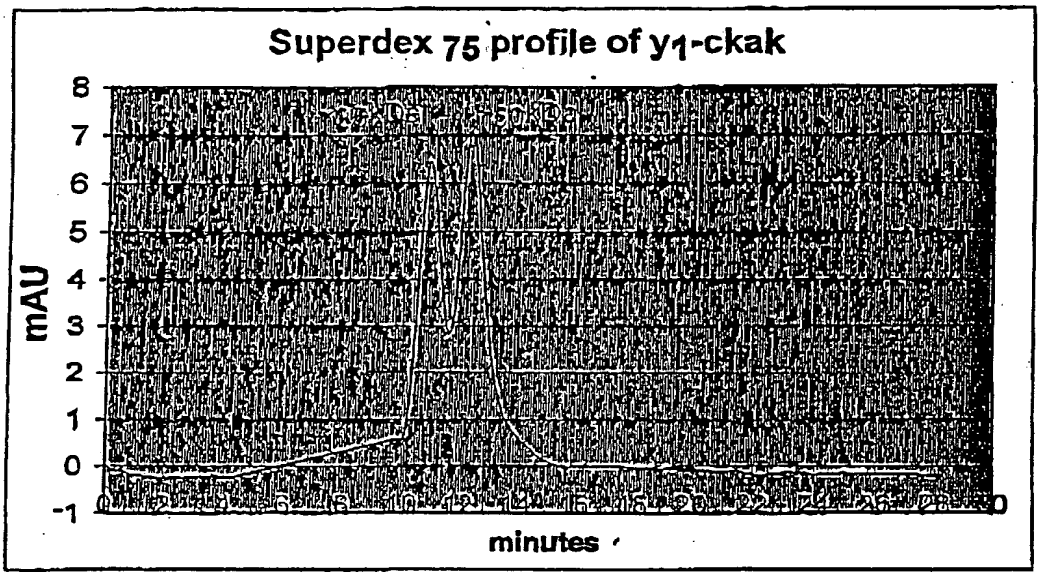


*FIG. 43*

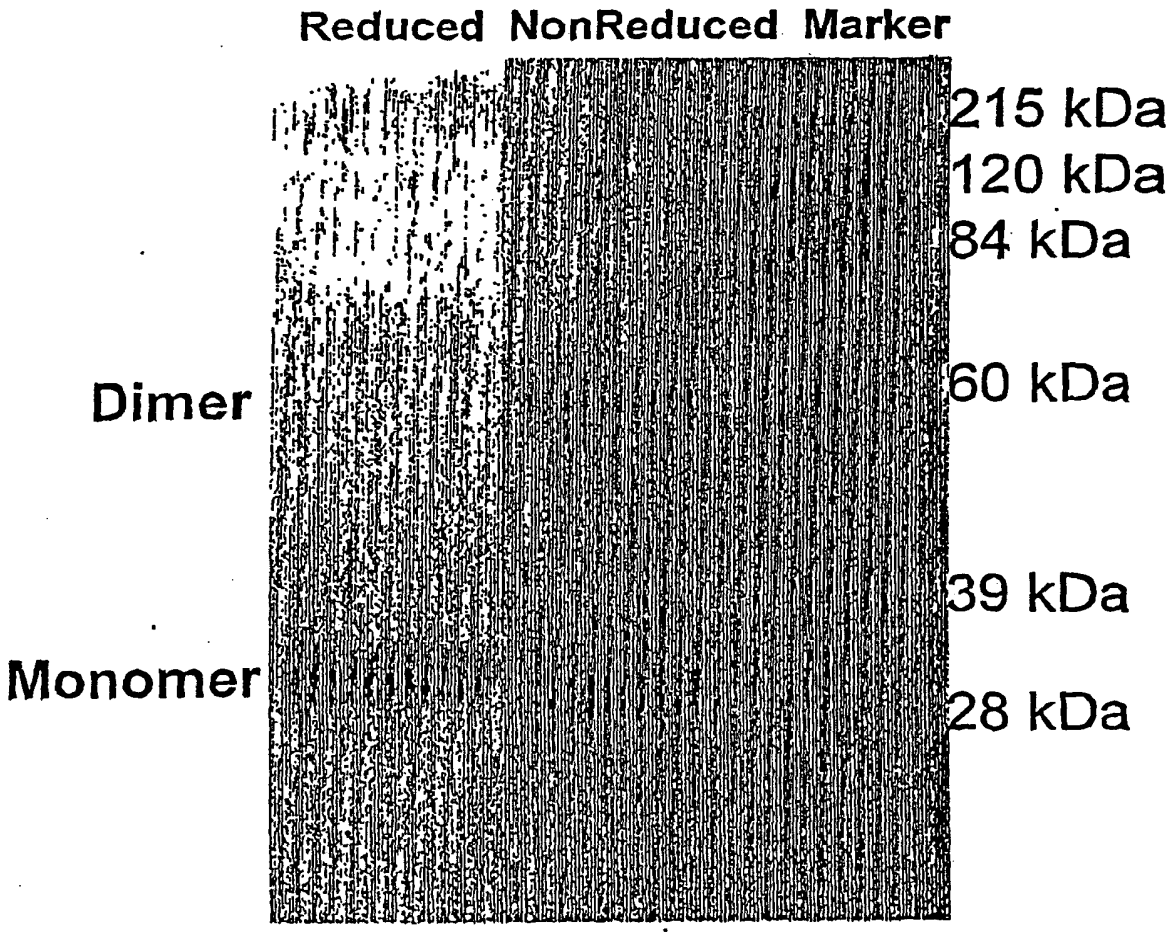
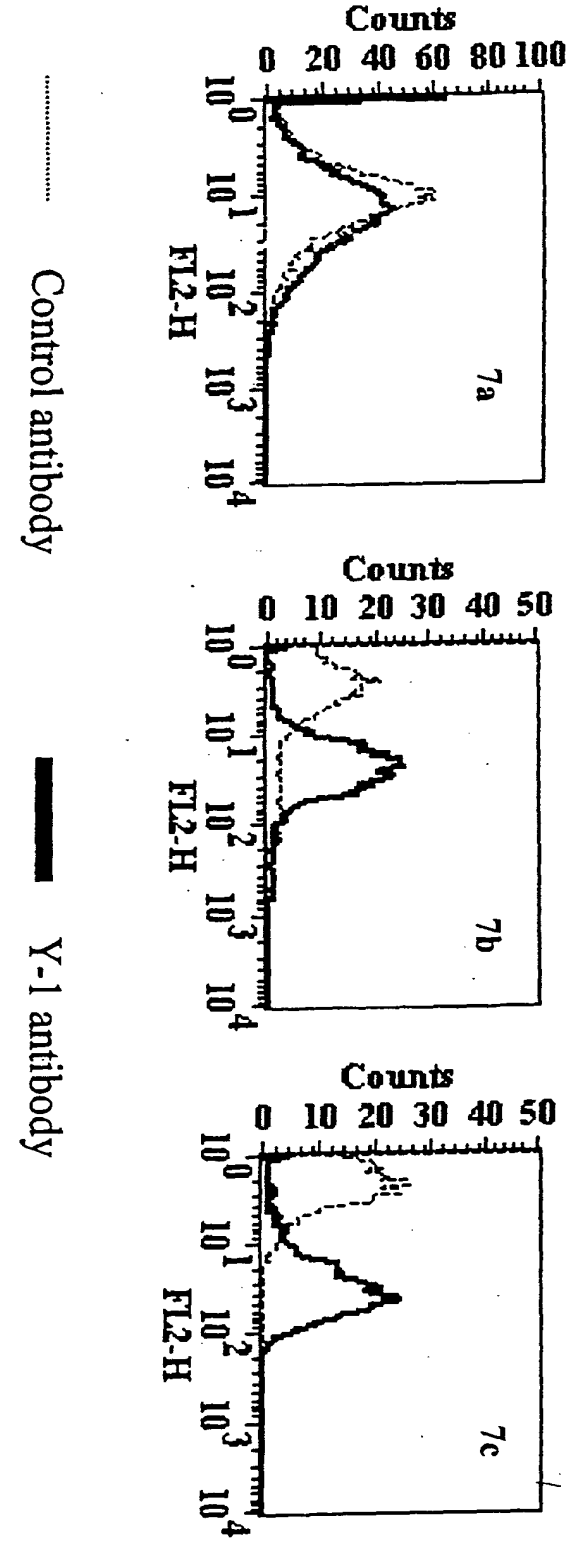


*FIG. 44*



# *FIG. 45*

## Epitopes of anti-GPIbα antibodies

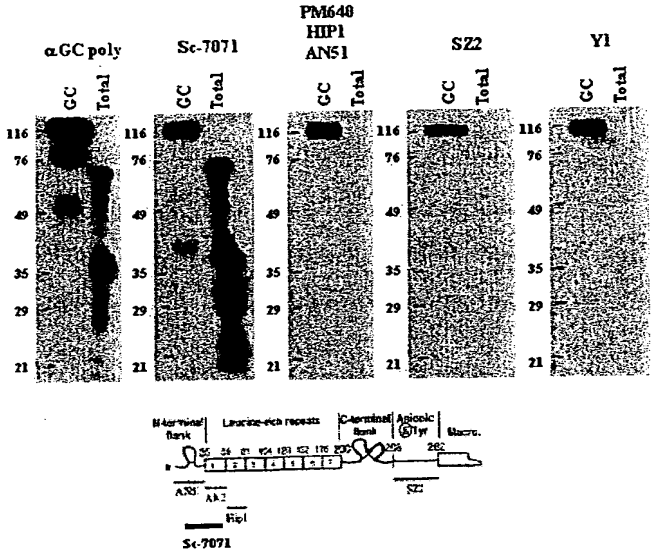


*FIG. 46*

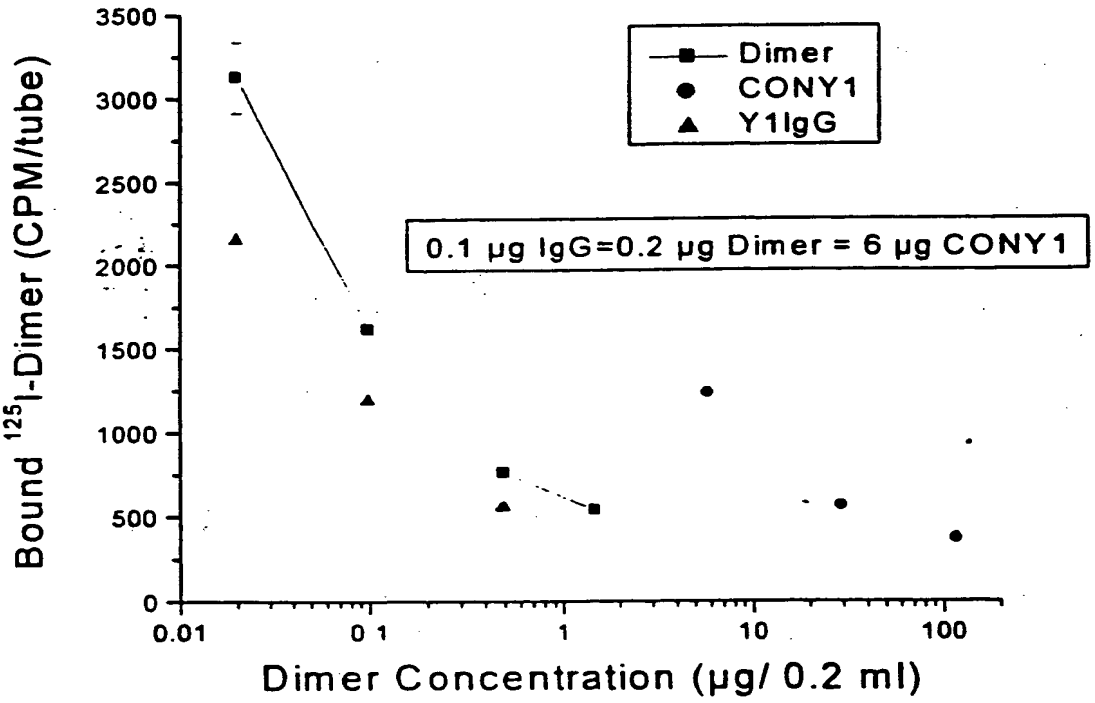
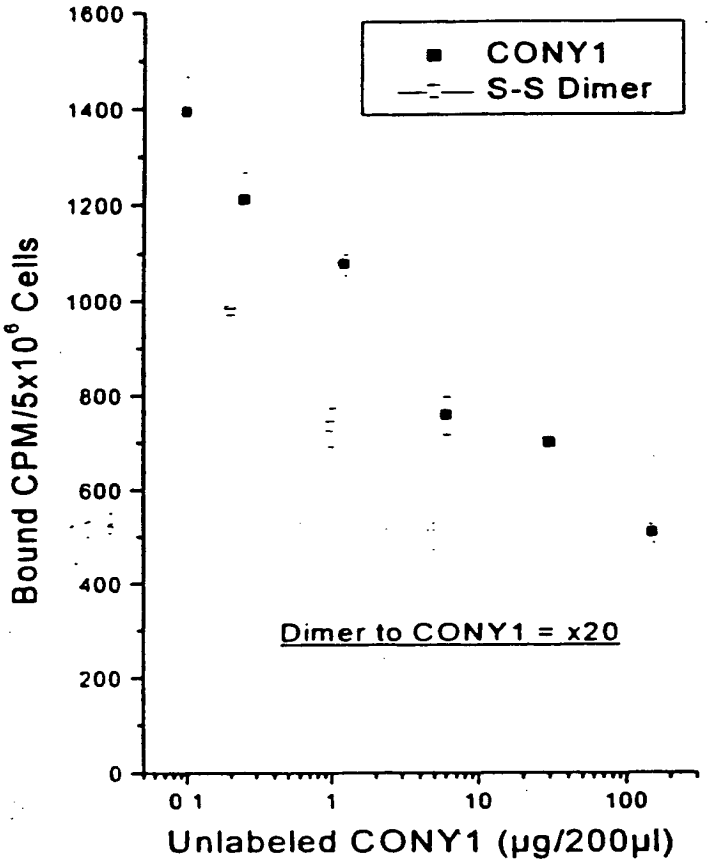


*FIG. 47*



## FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

**SEQ ID NO: 205 (nucleic acid sequence): SEQ ID NO: 206 (amino acid sequence)**

```
1     ATGGCCTGGGCTCTGCTGCTCCTCACCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1      M  A  W  A  L  L  L  L  T  L  L  T  Q  D  T  G  S  W  A  D

61    ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21     I  Q  L  V  E  S  G  G  G  V  V  R  P  G  G  S  L  R  L  S

121   TGTGCAGCCTCTGGATTCACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41     C  A  A  S  G  F  T  F  D  D  Y  G  M  S  W  V  R  Q  A  P

181   GGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61     G  K  G  L  E  W  V  S  G  I  N  W  N  G  G  S  T  G  Y  A

241   GACTCTGTGAAGGGCCGATTCACCATCTCTAGAGACAACGCCAAGAACTCCCTGTATCTG
81     D  S  V  K  G  R  F  T  I  S  R  D  N  A  K  N  S  L  Y  L

301   CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101    Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C  A  R  M  R  A

361   CCTGTGATTTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGCTTCCACCAAGGGCCCA
121    P  V  I  W  G  Q  G  T  L  V  T  V  S  S  A  S  T  K  G  P

421   TCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141    S  V  F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L  G

481   TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTG
161    C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L

541   ACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC
181    T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S

601   AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201    S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N

661   CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACT
221    H  K  P  S  N  T  K  V  D  K  R  V  E  P  K  S  C  D  K  T

721   CACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACTGTCAGTCTTCCTCTTC
241    H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F

781   CCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTG
261    P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V

841   GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281    V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E

901   GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301    V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V

961   AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC
321    S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V

1021  TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC
341    S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P

1081  OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
361    R  E  P  Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V

1141  AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381    S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S

1201  AATGGGCAGCCGGAGAACAACTACAAGACCACGTCTCCCCGTGCTGGACTCCGACGGCTCC
401    N  G  Q  P  E  N  N  Y  K  T  T  S  P  V  L  D  S  D  G  S

1261  TTCTTCCTCTATAGCAAGCTCACCGTGCACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421    F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F

1321  TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441    S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L

1381  TCTCTGGGTAAATGA
461    S  L  G  K  *
```

## FIG. 48B: The ORF and Amino Acid Sequnce of Y1-LC

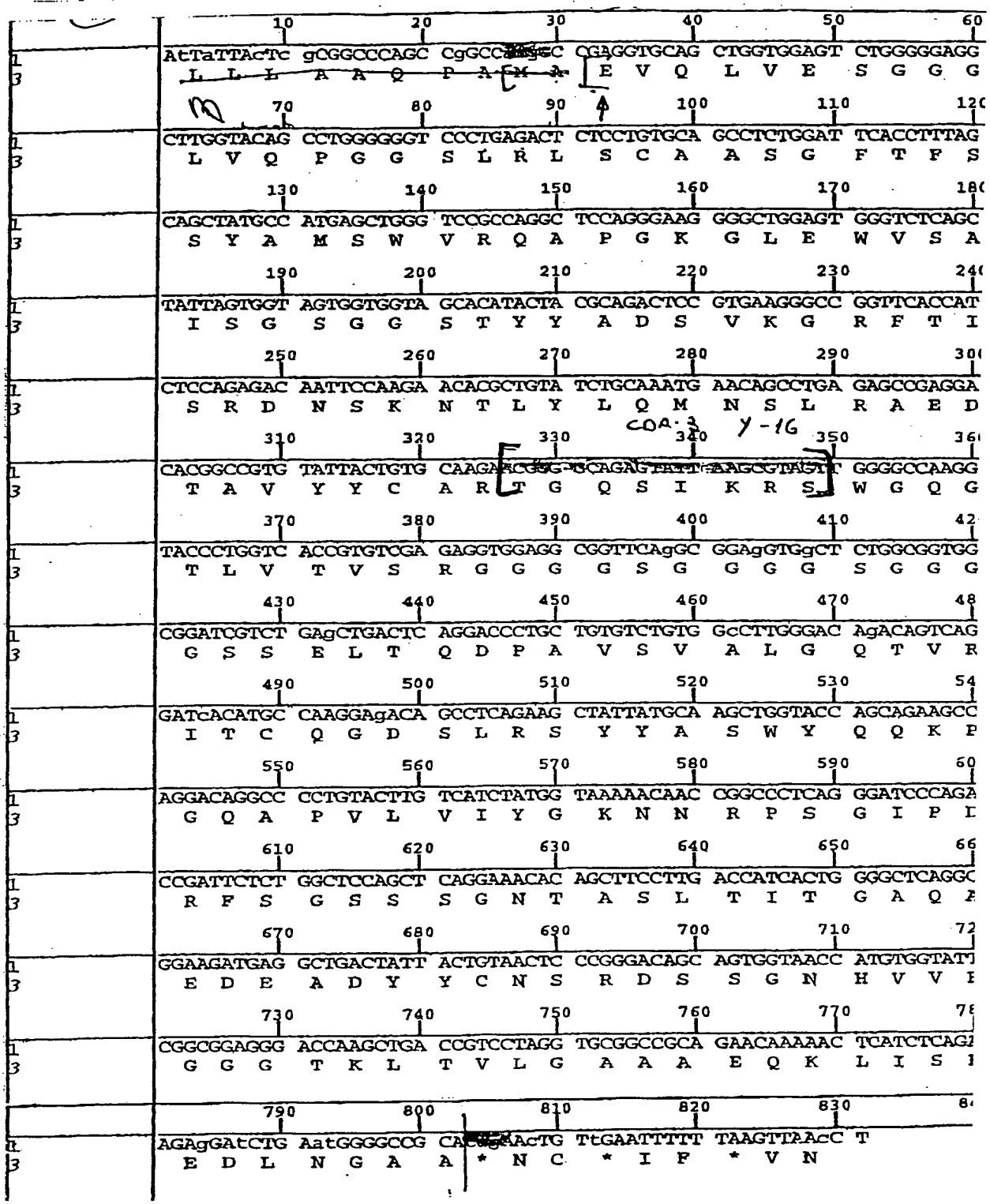
**SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)**

```
1       ATGGCCTGGGCTCTGCTGCTCCTCACCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1        M  A  W  A  L  L  L  L  T  L  L  T  Q  D  T  G  S  W  A  D

61      GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21       A  E  L  T  Q  D  P  A  V  S  V  A  L  G  Q  T  V  R  I  T

1212    TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41       C  Q  G  D  S  L  R  S  Y  Y  A  S  W  Y  Q  Q  K  P  G  Q

181     GCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTC
161      A  P  V  L  V  I  Y  G  K  N  N  R  P  S  G  I  P  D  R  F

241     TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81       S  G  S  S  S  G  N  T  A  S  L  T  I  T  G  A  Q  A  E  D

301     GAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGA
101      E  A  D  Y  Y  C  N  S  R  D  S  S  G  N  H  V  V  F  G  G

361     GGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121      G  T  K  L  T  V  L  G  Q  P  K  A  A  P  S  V  T  L  F  P

421     CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141      P  S  S  E  E  L  Q  A  N  K  A  T  L  V  C  L  I  S  D  F

481     TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161      Y  P  G  A  V  T  V  A  W  K  A  D  S  S  P  V  K  A  G  V

541     GAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181      E  T  T  T  P  S  K  Q  S  N  N  K  Y  A  A  S  S  Y  L  S

601     CTGACGCCTGAGCAGTGGAAGTCCCACAAAAGCTACAGCTGCCAGGTCACGCATGAAGGG
201      L  T  P  E  Q  W  K  S  H  K  S  Y  S  C  Q  V  T  H  E  G

661     AGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCATGA
221      S  T  V  E  K  T  V  A  P  T  E  C  S  *
```

# FIG. 49

```
    1           11          21          31          41          51
    |           |           |           |           |           |
  1 EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYAMSWVRQA PGKGLEWVSA ISGSGGSTYY   60
 61 ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARYA KTLMRQYSLW GQGTLVTVSR  120
121 GGGGSGGGGS GGGGSSELTQ DPAVSVALGQ TVRITCQGDS LRSYYASWYQ QKPGQAPVLV  180
181 IYGKNNRPSG IPDRFSGSSS GNTASLTITG AQAEDEADYY CNSRDSSGNH VVFGGGTKLT  240
241 VLGAAAEQKL ISEEDLNGAA
```

# FIG. 50



1/16 SEQ ID NO: 210

# *FIG. 51*

**Sequence of Y1-Biotag (SEQ ID NO: 211)**

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ

41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL

81 YLQMNSLRAE DTAVYYCARM RAPVIWGQGT LVTVSRGGGG

121 SGGGGSGGGG SSELTQDPAV SVALGQTVRI TCQGDSLRSY

161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA

201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG

241 GGLNDIFEAQ KIEWHE

# *FIG. 52*

Y1-cys-kak scFv (SEQ ID NO. 212)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGGSGGGG SSELTQDPAV SVALGQTVRI TCQGDSLRSY
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK